SCREENING OF *CARPOBROTUS EDULIS* EXTRACTS FOR ANTICANCER ACTIVITY AGAINST CERVICAL CANCER CELLS

BY

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Declaration

I, Walter Phogole, hereby, declare that the work submitted in this dissertation to the University of Limpopo for the degree of Master of Science in Biochemistry has not been previously submitted by me at this or any other University. This is my own work by design and in execution, and where use of others' work was utilised, it has been duly acknowledged.

Signature : ______________________________

Date : 12/09/2024

Dedication

This work is dedicated to almighty God, who is responsible for my existence and the strength He provided throughout the journey, also dedicate this work to all the women who lost their lives due to cervical cancer and the researchers who put effortless energy to investigate remedies to manage and cure the disease.

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Presentations and publications

Oral Presentation

- **W. Phogole**, V. Morafo and Z. Mbita. Screening of C*arpobrotus edulis* extracts for anticancer activity against cervical cancer cells. Faculty of Science and Agriculture Research Day 2023. University of Limpopo. 22 September 2023.

Abbreviations

A	
AA	Ascorbic acid
AC	Adenocarcinoma
AE	Acetone extract
AEL	Acetone extract leaves
AER	Acetone extract roots
B	
BCA	Bicinchoninic acid
BSA	Bovine Serum Albumin
C	
CEF	Chloroform/Ethyl-acetate/Formic acid
CIN	Cervical intraepithelial neoplasia
CO_2	Carbon dioxide
COX	Cyclooxygenase
Cr	Chromium
D	
DMSO	Dimethyl sulphoxide
DPPH	2,2-diphenyl-1-picryl-hydrazyl-hydrate
E	
EE	Ethanol extract
EEL	Ethanol extract leaves
EER	Ethanol extract roots

EGCG	Epigallocatechin gallate
EMW	Ethyl-acetate/Methanol/Water
F	
FBS	Foetal bovine serum
FRAP	Ferric reducing antioxidant power
H	
Hb	Haemoglobin
HIV/AIDS	Human immune virus/Acquired immune deficiency syndrome
HPV	Human papillomavirus
I	
IC_{50}	Minimum inhibitory concentration
J	
JE	Juice extract
L	
LC-MS	Liquid chromatography-mass spectrophotometry
LMICs	Low-middle income countries
LOX	Lipoxygenase
M	
MAPKs	Mitogen-activated protein kinases
MBC	Minimum bacterial concentration
MCH	Mean corpuscular haemoglobin

MCHC	Mean corpuscular haemoglobin concentrations
MIC	Minimum inhibitory concentration
MTT	3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide

N

NADPH	Nicotinamide adenine dinucleotide phosphate
NO	Nitrogen oxide

P

PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Polycythaemia
PGH2	Prostaglandin H2
PSN	Penicillin-streptomycin-neomycin

R

RBC	Red blood cells
RFLP	Restriction fragment length polymorphism
RIPA	Radio immunoprecipitation assay
RNS	Reactive nitrogen species
ROS	Reactive oxygen species

S

SCC	Squamous cells carcinoma
SEM	Standard error of the mean
SPF	Sun protection factor

T

TEA	Toluene/Ethanol/Ammonium hydroxide
TFC	Total flavonoids content
TLC	Thin-layer chromatography
TPC	Total phenolic content
TPTZ	Tripyridyl triazine

U

UHPLC-MS	Ultra-high performance liquid chromatography-mass spectrophotometry
UHPLC-UV/DAD	Ultra-high performance liquid chromatography-ultra-violet /Diode array detector
UNIN	University of the North
uPA	Urokinase type-plasminogen activator
USA	United States of America
UV	Ultra-violet

V

VEGF	Vascular endothelial growth factor

W

WE	Water extract
WEL	Water extract leaves
WER	Water extract roots
WHO	World Health Organization

Abstract

Introduction: Cancer is a primary cause of death and a significant impediment towards extending the life expectancy of human-kind. In terms of both incidence and fatality rates, cervical cancer is the fourth most common malignancy after lung cancer, breast cancer and colorectal cancer, worldwide. It is the second most common cancer and the primary cause of cancer fatalities in South African women. This cancer is primarily attributed to the cancer-causing Human papilloma virus (HPV). However, not all cases of cervical cancer are credited to this oncovirus. The soaring numbers can be ascribed to both inefficiencies associated with the current therapeutic strategies that are also related with adverse effects, and inadequacies linked to cancer diagnostic tools. Therefore, the development of novel anticancer medicines with improved efficacy and fewer side effects is critical. Thus, this study was aimed at evaluating the potential anticancer activity of *Carpobrotus edulis* extracts (*C. edulis*) against different cervical cancer cell lines.

Methodology: *C. edulis* extracts were screened for the presence of various phytochemicals using the standard conventional phytochemical tests. The potential antioxidant activities of the plant extracts were quantified using both the 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) and ferric reducing antioxidant power (FRAP) assays. The probable identities of the compounds found in *C. edulis* extracts was determined utilizing the Liquid Chromatography-Mass Spectrophotometry (LC-MS) for identification of compounds in *C. edulis*. The 3-(4,5-dimethyl-thiozol-2yl)-2,5-diphenyltetrazolim bromide (MTT) assay was utilized to assess the effects of the *C. edulis* extracts on HeLa, CaSki and non-cancerous (Hek-293) cell lines. To further validate the cytotoxic effects of the extracts (IC_{50}s) against the mentioned cells, the Muse™ Count & Viability assay was utilized. The Annexin V Apoptosis analysis was employed to investigate the potential cell death-inducing capacity of the *C. edulis* water extract against the cervical cancer cells. Furthermore, the proteome profiler human angiogenesis array was used to examine the potential effect of the water extract of *C. edulis* on angiogenesis in cervical cancer cell lines.

Results and Discussion: All the extracts were proven to possess various phytochemicals. *C. edulis* root extracts (acetone and ethanol) promoted the viability of cervical cancer cells while the aqueous extract had reduced the viability of both HeLa (IC_{50} = 1000µg/mL) and CaSki (IC_{50} = 1000µg/mL) cells but showed no effect against the non-cancer cells (Hek293).. The *C. edulis* aqueous extract induced minimal apoptosis in both CaSki (7.950 ± 1.422) and HeLa (8.933 ± 0.361) cells studied. *C. edulis* root water extract (1000 µg/ml) treated cells had higher levels of urokinase type-plasminogen activator (uPA), and vascular endothelial growth factor (VEGF) was somewhat higher in *C. edulis* root water extract treated cells than in curcumin-treated and untreated cells.

Conclusion: The *C. edulis* root water extract was found to have potential anticancer effects on HeLa and CaSki cells, but safe against non-cancerous Hek-293 cells. Extracts from the roots of *C. edulis* in acetone or ethanol did not have any cytotoxic effects on HeLa or CaSki cells. HeLa or CaSki cell death were unaffected by the *C. edulis* root water extract, while VEGF and uPA protein levels, which promote angiogenesis, were modestly elevated.

Key words: *Carpobrotus edulis*, phytochemicals, cervical cancer, apoptosis, angiogenesis, proteins.

Chapter One: Introduction

1. Introduction

Worldwide, cancer is among the leading causes of death in many nations (Yingchoncharoen, 2016). All malignancies, in general, involve the body's unregulated generation of new, defective, or aberrant cells (Harrington *et al.,* 2023). As human populations expand, get older, and adopt lifestyle choices that raise their risk of cancer, cancer incidence diagnoses and deaths is anticipated to increase quickly, adding to the already heavy burden (Torre *et al.,* 2015). One of the factors causing this global shift is genetic instability, which is defined as circumstances that may result in spontaneous alterations in the genome that may be either short-term or long-lasting (Makgoo, 2019).

The need for anticancer treatments is growing (Unno *et al.,* 2005). The usage of laboratory animals is decreased by using in vitro cytotoxicity screening methods (Abraham *et al.,* 2004). As a result, more cultured cell and tissue specimens are being used (Thampi *et al.,* 2015). In South Africa, cancer has become a recognised health issue having a significant morbidity and death rate from cervical cancer (Botha *et al.,* 2017). South Africa has 32 Incidents of cervical cancer among 100,000 women, having the greatest age standardised prevalence of the disease worldwide (Ferlay *et al.,* 2018).

1.1. Cervical cancer

Cervical cancer is an aggressive malignancy located in the uterus that it is divided into two histopathological forms: squamous cells carcinoma (SCC) and adenocarcinoma (AC) [Torre *et al.,* 2015]. The incidence rate of SCC is 70% worldwide, making it more prevalent (Herrero et al., 2015) as compared to AC. While AC is produced by glandular cells lining the cervix's canal (the endocervix), SCC is formed by squamous cell carcinoma surrounding the exterior area of the cervix that exits to the end of the ectocervix [Hull *et al.,* 2020]. The common of cervical tumours begin in the transformation zone, which is the area containing the squamous cells and thin, flat glandular cells (Waggoner, 2003). The higher incidence of cancer in developing nations like South Africa can be attributed to several factors such as delayed diagnosis, advanced phases and infections with human papillomavirus (HPV) at the time of diagnosis, insufficient treatment availability or accessibility, insufficient treatment facilities, and logistical and cultural barriers to treatment (Hull *et al.,* 2020) which make the prognosis dismal (Torre *et al.,* 2017).

Cervical cancer is treated depending to the stages (Lorusso *et al.,* 2014). Patients who have locally more severe forms of cancer respond best to combined chemotherapy and pelvic irradiation; however, those with earlier stages may still be treated with radiotherapy or surgery (Kang *et al.,* 2015). Patients with recurring or chronic diseases and those with metastatic tumours have few therapy choices despite advancements in medical science (Pfaendler and Tewari, 2016). For treatment of severe and metastatic cervical tumours, several chemotherapeutic drugs have demonstrated efficacy, comprising of cisplatin (Leisching *et al.,* 2015), topotecan (Muderspach *et al.,* 2001), ifosfamide (Downs *et al.,* 2011), paclitaxel (Wang *et al.,* 2015) and carboplatin (Katanyoo *et al.,* 2011). For metastatic cervical cancer, cisplatin has been reported to be among the most effective of these chemotherapy drugs (Lorusso *et al.,* 2014) and the success of the medication is due to the formation of adducts by the drug and DNA that obstruct gene transcription, oxidative stress caused by oxygen, and tumour degeneration of cells (Oz *et al.,* 2015). Nevertheless, tumour resistance and severe side effects such as (thrombocytopenia, neutropenia, nephrotoxicity, neurotoxicity, and anaemia from haematological toxicity) restrict its clinical usage (de Freitas *et al.,* 2014) as well as bone marrow depression (Das *et al.,* 2014).

Radiation and chemotherapy are not only effective against cancer cells, they also have the potential to eliminate healthy cells as well (Chakraborty, 2019). Thus, effective medicines and improvements are needed to treat aggressive and resistant cervical cancer without compromising healthy cells (van de Vooren *et al.,* 2014). Numerous extracts from plants and phytoconstituents were investigated in relation to cervical cancer cells. As a result, research into developing novel anti-cancer medications with increased effectiveness and less adverse effects has persisted (Hosseini and Ghorbani, 2015). According to research, dietary phytochemicals such as flavanols have an impact on chemotherapy treatment and can help cancer patients recover (Kashafi *et al.,* 2017). Thus, therapeutic and commercial applications for medicinal plants remain paramount (Booth *et al.,* 2012).

1.2. Medicinal plants

South Africa showed to have a long history of using traditional medicine (Street et al., 2008) through the use of traditional healers since early civilisation, and these healers and the use of herbal remedies continue to have been used in communities (Odeyemi and Bradley, 2018). In 2018, it was reported that approximately 27 million South Africans relied on traditional medicine as their primary source of health treatment requirements (Khan, 2014; Oguntibeju, 2018, Tungmunnithum et al., 2018). For many members of the communities, the potential benefits of medicinal plants have been passed from generation to generation (Marrelli, 2021); however, these plants' mechanism of action has been a subject of many research endeavours.

Throughout the main healthcare systems of underdeveloped nations, conventional medication is the most conveniently available and reasonably priced treatment option (Mudimba and Nguta, 2019). Various medicinal herbs were used in traditional therapies, and some anticancer treatments are made from medicinal herbs, spices, and vegetables (Wang *et al.,* 2013). The bioactivity of medicinal plants is due to presence of phytochemicals that are produced to serve as a line of defence against pathogens and they were proven to offer health benefits (El-Saadony *et al.,* 2021). Considering organic products in the current medical system, phytochemicals that are effective are present in around 25% of medications (Wang *et al.,* 2013). Clinical trials are investigating the effectiveness of plants and their active ingredient in the therapy for cervical cancer, which is closely related regarding the early lesions of the cervical cancer screening program (Chakraborty, 2019).

The severely deteriorating prognosis of artificial medicines led to the beginning of the search for natural alternatives to treat cervical cancer (Wang *et al.,* 2008). Discovery of potentially active plant-based products targeting cervical cancer could go a long way for potential therapeutic use (Chakraborty, 2019). Furthermore, because herbal medicines have been shown to trigger apoptosis (Lee *et al.,* 2002), control the DNA repair process (Ghagane *et al.,* 2017) and suppress the enzymatic processes and hormones required for tumour growth which are essential for slowing down the development of cancer (Russo *et al.,* 2019).

1.3. *Carpobrotus edulis*

Carpobrotus edulis (*C. edulis*), is a succulent herbal plant with medicinal and edible qualities (Akinyede *et al.,* 2020). Numerous research endeavours have been carried out on various extract types, taking into account their phytochemical profile and their biological function, in an effort to confirm the potential pharmacological effects of *C. edulis* and to explain its traditional usage (Bazzicalupo *et al.,* 2021). The distinct parts of *C. edulis* were employed for numerous medicinal purposes. The sap of the leaves can be used as an astringent to treat skin ailments, to treat gastrointestinal and inflammatory diseases (Mudimba and Nguta, 2019). The leaves and roots of *C. edulis* have been used as antiviral medications to treat disorders associated with Human immunodeficiency virus (HIV) infection also the virus itself (Omoruyi *et.al.,* 2012). The past study has confirmed its antioxidant (Ibtissem *et al.,* 2012), antibacterial (Martins *et.al.,* 2011), anticholinesterase (Custódio *et al.,* 2012) and immune-modulating properties (Ordway *et al.,* 2003) from a scientific standpoint.

Additionally, *C. edulis* has been shown to have anticancer activities on mouse-lymphoma cells, through p-glycoprotein suppression serving as a reported mode of activity (Martins *et al.,* 2010). It was demonstrated that the water-based extract together with ethanol and water (1:1 v/v) herbal extract caused cytotoxicity and inhibited glycation on HCT-116 human colonic tumour cell lines (Hafsa *et al.,* 2016). In human neuroblastoma cell lines, the aqueous extract of C*arpobrotus edulis* exhibited antiproliferative properties (Omoruyi *et al.,* 2021). Thus, this study will focus on evaluating the effects of *C. edulis* extracts (Acetone, Ethanol and Water) on cervical cancer cell lines since there are no studies published on the effects of *C. edulis* against cervical cancer.

1.4. Problem statement

Cancer is the leading cause of mortality and substantial hindrance towards increasing the longevity of life in human-kind (Sung *et al.,* 2021). Based on the World Health Organization report (WHO, 2020), cancer was the primary reason of mortality in ages below 70 in 112 of 183 nations. Cervical cancer was the fourth most common malignancy after lung cancer, breast cancer and colorectal cancer, with regard to each incidence and mortality or death rates, worldwide (Bray *et al.,* 2018). In South Africa, cervical cancer is the second prevalent malignancy in women and the leading cause of cancer mortality (Hoque *et al.*, 2014; Akokuwebe *et al.,* 2021). Cervical cancer is primarily attributed to cancer-causing Human papilloma virus (HPV) specifically HPV16 and HPV18 (Simms *et al.,* 2020) however not all cases are credited to this oncovirus. The current therapeutic strategies are associated with adverse effects; therefore, the establishment of novel anticancer medicines with enhanced effectiveness and less side effects is critical (Gutheil *et al.,* 2012). Some findings have pointed out that phytochemicals from medicinal herbs showed to exhibit cell anti-proliferative qualities, and thus represent indicating leads for the development of novel therapeutic anticancer agents (Rao *et al.,* 2016). The bioactive compounds derived from various parts of medicinal plants have significantly contributed to the development of the currently used anticancer drugs and more plants need to be screened for novel antineoplastic drugs (Anand *et al.,* 2019). *Carpobrotus edulis (C. edulis)* is among the plants with medicinal value; however, its anticancer activity has not been studied against cervical cancer.

1.5. Research question

Does *C. edulis* extracts have anticancer activity against cervical cancer cell lines?

1.6. Aim of the study

To evaluate the potential anticancer activity of *Carpobrotus edulis* extracts against different cervical cancer cell lines.

1.7. Objectives

The objectives of the study were to:

i. analyse the phytochemical content of *Carpobrotus edulis* extract using thin layer chromatography, different qualitative and quantitative phytochemical assays;
ii. assess the effect of *Carpobrotus edulis* extracts on proliferation and viability of cervical cancer cell lines by using 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT assay*)*;
iii. investigate potential apoptotic activities of *C. edulis* extracts against cervical cancer cells by employing Annexin V analysis assay, and;

iv. evaluate the effects of *Carpobrotus edulis* extracts on human angiogenesis proteome profiler.

1.8. Organisation of the thesis

The dissertation is divided into five chapters, sources and appendices. The contents of each chapter are explained below.

1.8.1. Chapter One

This chapter discusses the problem statement, emphasizing the shortcomings of the present cervical cancer treatment and the potential benefit of using *C. edulis* for the treatment of cells. It also covers aim, objectives, and questions to be addressed.

1.8.2. Chapter Two

This chapter reviews the current state of the field of molecular biology, discrepancies in understanding regarding the significance of *C. edulis* effects on cervical cancer, and the plant's antioxidant, antimicrobial, antidiabetic, anticancer, anti-inflammatory, photoprotective activities, and chromium VI activity.

1.8.3. Chapter Three

Chapter 3 details all of the materials and techniques are that were used in this study. This chapter is associated by appendices which detail information about the manufacturer of kits used and the composition of the buffer solutions utilized.

1.8.4. Chapter Four

This chapter covers the results of this obtained from this project, detailing on the antioxidant activity of *C. edulis* extracts, their cytotoxic effects on Hek-293, HeLa, and CaSki cells, their apoptotic effects, and their potential role in inhibiting angiogenesis.

1.8.5. Chapter Five

Chapter 5 provides the results obtained. It also summarizes conclusions and provide potential directions for future research.

1.8.6. References

The sources that were used in this dissertation to bolster the scientific claims made in this study.

1.8.7. Appendices

Appendices are listed under this section.

2. Introduction

Cancer is one of the leading causes of death and a key barrier to increasing the average lifespan in every country on the entire globe (Bray *et al.,* 2021). In accordance with projections of the World Health Organization (WHO) for 2019, cancer ranks first and second in 112 out of 183 nations for deaths occurring before the age of 70, and in a further 23 countries, it ranks third or fourth (WHO, 2020). Globally, there were projected to be 19.3 million new instances of cancer in 2020 (excluding non-melanoma skin cancer, or 18.1 million), and around 9.9 million cancer-related deaths were projected (Sung *et al.,* 2021). Worldwide, there were approximately 570 000 instances reported and around 311 000 deaths due to cervical cancer in 2018, this makes it the fourth highest prevalent malignancy amongst females (Gultekin *et al.,* 2020). Africa has the greatest regional incidence and fatality rates, which are seven to ten times greater than those in the developed nations (WHO, 2018). Currently, low- and middle-income nations account for around 90% of all fatalities (WHO, 2020). Cervical malignancy is a medical condition that represents disparities across various groups with regard to the accessibility of a nationwide immunization program, population based cervical carcinoma evaluation and access to high-quality treatment (Ginsburg *et al.,* 2017). In South African women, cervical cancer ranks as the fourth most prevalent malignancy diagnosed (WHO, 2020).

2.1. Cervical cancer causes

There are two main histological kinds of cervical cancer: adenocarcinoma (approximately 25%), which originates within the glandular columnar stratum of the endocervix, and squamous carcinoma (about 75%), which mostly begins at the transforming zone of the ectocervix (Ngoma and Autier, 2019). Cervical neoplasia is a term used to characterise abnormal cells seen on the cervix's surface during a biopsy and generally caused by human papillomavirus (HPV), this occurs in 99.7% of cervical cancer occurrences (Walboomers *et al.,* 1999). Most HPV infections remain temporary and will disappear on themselves. Persistent infection; however, may occasionally lead to the precancerous diseases intraepithelial neoplasia of the cervical or adenocarcinoma (Small *et al.,* 2017). For majority of the women, the progression from dysplasia to destructive cancer may take years or even decades if therapy is not received. Nonetheless, this change might happen in less than a year in around 10% of individuals (Kessler, 2017). Furthermore, it appears that adenocarcinoma in situ is more challenging to identify with Pap smear (Papanicolaou tests), which may contribute to the rising frequency associated with this variant of cervical cancer (Fujiwara *et al., 2014*).

There are several reasons that have were recommended to increase the menace of developing a persistent infection and the subsequent malignant transformation (Gershenson *et al.,* 2004). These factors include using oral contraceptives for an extensive amount that passed, smoking cigarettes, having high number, and mutual infection with either the human immunodeficiency virus or type 2 herpes simplex virus (Fujiwara *et al.,* 2014). The two most significant factors of these are tobacco use and high parity (Castellsagué and Munoz, 2003) since they promote the course of high-rate HPV infection (Chatzistamatiou *et al.,* 2013) and lead to high incidences of cervical precursors to cancer and malignancy (Pista *et al.,* 2011). The most prevalent HPV strains among women having cervical carcinoma, in descending order of frequency, are 16, 18, 45, 31, 33, 52, 58, and 35 (Lee *et al.,* 2016). It is estimated that HPV strains 16 and 18 accounting for about 70% of cases (Small *et al.,* 2017).

2.1.1. Epigenetic factors

It has also been shown that suppression of immunity, dietary difficulties (Torre *et al.,* 2015) and concurrent infections with the virus that causes herpes simplex type-2 all contribute to the onset and spread of cervical cancer in women (Hull *et al.,* 2020). A number of immunological and genetic host and viral variables are also anticipated to have a role (Muñoz *et al.,* 2006). More than 80 environmental chemicals have been found by (Goodson *et al.,* 2015) to have a part in improper regulation of host mechanisms during cancer. In addition, substances linked to smoke from cigarettes, including tar from coal by-products, vaginal tar-based hygiene goods, and smoking breathed from biomass-burning ovens, are ecological contributors to risk for cervical cancer (Hull *et al.,* 2020). In low-income nations, these environmental variables have the ability to trigger the signalling pathways that lead to the development of HPV-related malignancies, such as SCC (Haverkos, 2005). Similar to the pathways identified as among the hallmarks of cancer, these pathways involve advanced inflammation, hyper-proliferation signalling, inability of growth-factor communications, deception of cell death, persistent angiogenesis, genomic instability, mutation, and disturbance of normal metabolic processes (Goodson *et al.,* 2015; Hull *et al.,* 2020).

2.2. Diagnosis and prevention of cervical cancer

The widespread availability of screening programs that enable the early detection and excision of precancerous lesions accounts for a portion of the geographical heterogeneity in cervical cancer incidence (Hull *et al.,* 2020). Cervical cancer has several complex and regionally variable causal factors, one of which is infection with HPV (Scarinci *et al.,* 2010).

2.2.1. Cervical cancer screening

Reducing and eliminating the high death rate and number of new instances of cervical cancer due to ongoing HPV infection is one of the foremost worldwide concerns. Precisely, this occurs throughout the HIV testing phase, a Pap smear check for cervical abnormalities is strongly advised (Hull *et al.,* 2020). Women who have already undergone screening and received a negative diagnosis should undergo this process again every three years (Chatterjee *et al.,* 2016). It is anticipated that routine cervical cancer screening for women will reduce their lifetime chance of contracting the illness (Goldie *et al.,* 2005).

Cervical cancer population screening in low-income and resource-constrained areas is still elusive (Future II Study Group, 2007). In Southern Africa, the percentage of women who have cervical cancer screenings varies from 4.1 to 38.0% (Cronje and Beyer, 2007). According to research, out of 580 females from villages, 9.6% had received an HPV screening, compared to 17.3% in 642 females across urban regions (Gakidou *et al.,* 2008). There are a number of analyses for this poor coverage, involving inaccessibility, a shortage of financing, low community knowledge and cost-effectiveness (Jit *et al.,* 2014), Pap smear problems (Garland *et al.,* 2007), a lack of focus on communal strategy, or the application of inefficient public programs (Wake *et al.,* 2009).

In low-middle income countries (LMICs), it is projected that the incidence of HIV-associated cervical cancer would gradually increase over the next ten years [Gopal *et al.,* 2014]. Even though cervical cancer is less common in LMICs than in many industrialized nations, the incidence of cancer in these areas is rising as a result of late stages of the disease at diagnosis and limited access to therapy (Jacobson *et al.,* 2018). Consequently, rapid intervention is recommended in the disciplines of public also global health (Herrero *et al.,* 2015; Brüggmann *et al.,* 2018). Detection of precancerous lesion effectively was attained with 62.5 and also HPV mutual infection finding was 25% respectively, utilizing the PapilloCheck® microarray as well as the HPV restriction fragment length polymorphism (RFLP) PCR test (Golfetto *et al.,* 2018). The PapilloCheck® microarray analysis is thought to be an extremely sensitive and informative test, however the RFLP PCR assay is recommended as the preferred HPV test for the screening at LMICs due to its cost-effectiveness (Golfetto *et al.,* 2018). Meanwhile, it is still difficult to provide population-level screening at a large scale in LMICs.

2.2.2. Cervical cancer prevention

Around 1.7 million cases of cervical cancer were identified in 2010 (Gelband *et al.,* 2016); Adesina *et al* projected in 2013 about 11 million women throughout Africa's Sub-Saharan region will receive a diagnosis during the following 10–20 years. An effective control strategy for preventing cervical cancer is crucial, but the treatment can also help (Goss *et al.,* 2013). It is been hypothesized that an affordable HPV vaccine can save countless lives of girls aged 9 to 12 (Chatterjee *et al.,* 2016) and after 50 years of widespread vaccination, it is anticipated that the number of instances of cervical cancer would significantly decline (Jit *et al.,* 2014). The two main HPV subtypes (low risk and high risk) that are targeted by vaccination are HPV 6, 11, 16, and 18 strains are targeted by the quadrivalent vaccination (Schiller *et al.,* 2012), whereas the HPV 16 and 18 strains are targeted by the bivalent vaccination (Herrero *et al.,* 2015).

Cervical intraepithelial neoplasia (CIN) has demonstrated to be prevented by HPV vaccinations (Joura *et al.,* 2015), which also successfully help to prevent HPV infection (Garland *et al.,* 2007). Due to a drop in cost, HPV vaccines have become more and more common in middle-income nations since 2011 (Herrero *et al.,* 2015). From 2012, low-income nations have been able to implement vaccination campaigns because of increasing financial assistance for HPV vaccine projects (Levin *et al.,* 2014). In South Africa, the National HPV Vaccination Program initially offered HPV vaccine to women in 2014 (Kocjan *et al.,* 2015). The main targeted age of HPV immunisation is 9 years old (learners in grades 3 and 4) in South Africa (Hull *et al.,* 2020).

The European Medicines Agency states that, depending on whether a girl has received their Gardasil or Cervarix vaccination, the usual dosage for the child's age is two administrations between 0 and less than 24 weeks (Nakisige *et al.,* 2017). As stated in clinical research by Herrero *et al.* (2015), women between ages of 15 and 26 who weren't subjected to the virus before can receive three doses of the HPV vaccine and be completely protected against lesions that are precancerous and ongoing HPV infection. To lower the occurrence, workload, and death rates of cervical cancer, treatment (or vaccine) and screening are essential (Ghebre *et al*., 2017).

2.3. Role of medicinal plants in cervical cancer treatment

Since traditional medicine is still widely available, affordable, and in line with cultural beliefs, 65-80% of people in underdeveloped nations still use it as a primary source of healthcare (Chakraborty, 2019). The therapeutic effectiveness of plants and their constituents in treating cervical cancer is being investigated and these studies must be conducted in conjunction with a screening program aimed at identifying the disease's early lesions (Kma, 2013). Secondary metabolites called phytochemicals are in command of many of the actions frequently connected to the application of medicinal herbs for management, therapy, and alleviation of a wide range of illnesses, including fatal conditions (Akinyede *et al.,* 2020). These substances are biologically active and have antibacterial and antioxidant effects, as well as modulating detoxification enzymes, boosting the immune system, reducing platelet aggregation, modifying hormone metabolism, and having anticancer capabilities (Koche *et al.,* 2016).

2.3.1. Classification of phytochemicals

Important medicinal plants with pharmacological properties that arise from the build-up of bioactive phytochemicals in plant tissue, which are referred to as both primary and secondary metabolites (Chanda and Ramachandra, 2019). The organic substances that are considered primary metabolites, that have been beneficial to the growth along with the development of humans, which incorporates glucose, starch, polysaccharides, proteins, lipids, and nucleic acids (Egamberdieva *et al.,* 2017). The secondary metabolic compounds formed by herbs include volatile oils, flavonoids, alkaloids, terpenoids, saponins, steroids, glycosides, tannins, are among others (Shakya, 2016).

Secondary metabolites, which contain phytochemicals and other pharmacologically active substances, have therapeutic utility and can help to manage variety of ailments (Chanda and Ramachandra, 2019). These secondary metabolites consists of Alkaloids, which have antispasmodic, anti-malarial, analgesics and diuretics properties(Egamberdieva *et al.,* 2017); Terpenoids, which have anti-viral properties, anthelmintic, antimicrobial, anticancer, antimalarial properties, and antiinflammation properties (Wink, 2015); Glycosides, which are known for their anti-fungal properties and anti-microbial properties (Mamta *et al.,* 2013); Flavonoids and phenols, which remain to have antiallergic, antioxidants, and antimicrobial properties (Shakya, 2016) and saponins, which possess anti-inflammation properties, anti-viral, and plant defence properties (Mamta *et al.,* 2013).

2.4. *Carpobrotus edulis* distribution and survival

Carpobrotus edulis (*C. edulis*), is a succulent herbal plant with both medicinal and culinary uses which is a member of the Aizoaceae family, that remains to be among the utmost abundant and diverse plant family in South Africa and the one that have gotten relatively little consideration in terms of its potential medical applications (Akinyede *et al.,* 2022). In addition to being widely distributed along other coastal African countries along with additional continents, *C. edulis* is an aerotolerant halophyte that is frequent mostly in Western and Eastern Cape areas of South Africa (Omoruyi *et al.,* 2012).

2.4.1. Taxonomy of *Carpobrotus edulis* classification

The domain of Eukaryota includes the Kingdom; Plantae, Phylum; Spermatophyta, Subphylum; Angiospermae; Class; Dicotyledonae, Order; Caryophyllales, Family; Aizoaceae, Genus; Carpobrotus, and Species; *Carpobrotus edulis* (Akinyede *et al.,* 2022).

2.4.2. The *Carpobrotus edulis* plant's phytochemistry

For the treatment of numerous chronic diseases, including cancer, natural products, particularly plant-based secondary metabolites known as phytochemicals, have been used for many years (Shukla *et al.,* 2015). Secondary metabolites known as phytochemicals are in charge of the numerous functions frequently connected with using medicinal plants for treating, mitigating, and managing a varied range of ailments, including life-threatening diseases (Akinyede *et al.,* 2020).

2.4.2.1. Qualitative analysis of *C. edulis*

The following phytochemical groups were discovered through phytochemical screening of different parts of the *C. edulis* plant: chlorides, saponins, sulphates, alkaloids, flavonoids, coumarins, anthraquinones, cardiac glycosides, cyanogenic glycosides, tannins, unsaturated sterols, carbohydrates (Mudimba *et.al.,* 2019). The flowers were found to have the highest overall flavonoid content and to be highly polyphenolic based on analysis of phytochemicals compared to (Eman, 2011) whereas the leaves had the highest tannin content (Van Der Watt and Pretorius, 2001).

2.4.2.2. Quantitative analysis of *C. edulis*

The amount of total polyphenols in the leaves of *C. edulis* differs greatly from other plant parts (Mudimba *et al.,* 2019). There is no specific solvent which is recognised to isolate all the biomolecules from the plant on its own due to the greatly varying types of phytochemical components present in plants (Ordonez *et al.,* 2006). Hexane, water, ethanol, and acetone were important solvents during the quantitative evaluation for various polyphenols isolated from plant specimens according to their polarities the biomolecules possess (Omoruyi *et al.,* 2012). This had been proved that these active ingredients, including flavonoids, have antimicrobial properties (Martins *et al*., 2005), neuroprotective effects (Custódio *et al.,* 2012) and anticancer activities (Hafsa *et al.,* 2016).

When the phytochemical concentrations were analysed, it was discovered that the *C. edulis* leaf extract had phenolic content, flavonoid content, and tannin content reported by (Omoruyi *et al.,* 2012). In the solvents, acetone, hexane, and ethanol, respectively, the highest concentration was found in the phenolic content (Castañeda-Loaiza *et al.,* 2020). Moreover, a comparable investigation on the fruit's peel and flesh revealed that the peel's phenolic content was 272.83 mg TE/g, its flavonoid content was 1.58 mg TE/g, and its tannin content was 20.3 mg/g, with the highest concentrations emerging from the ethanol solvent alone when compared to other solvents (Omoruyi *et.al.,* 2012). As stated by (Chokoe *et al*., 2008), *C. edulis* leaves gathered in the autumn season had greater quantities of these phytochemicals despite being a crude extract compared to other plant components.

2.4.3. Biomedical activities of *Carpobrotus edulis* extracts

C. edulis' in vitro biological activity demonstrated the plant's antioxidant (Falleh *et al.,* 2011), antidiabetic (Castañeda-Loaiza *et al.,* 2020), photoprotective (Lassoued *et al.,* 2021), anticancer (Hafsa *et al.,* 2016), antimicrobial (Martins *et al*., 2005), anti-inflammatory properties (Ondua *et al.,* 2019) also activity against chromium vi (Obaseki *et al.,* 2021).

2.4.3.1. Antioxidant properties of *C. edulis*

Polyphenols are major plant compounds that are generally recognised to have a wide range of biological consequences, including antioxidant properties. Scientists, food manufacturers, and consumers are all becoming increasingly interested on the role of antioxidant component in plant products play in sustaining health and prevention of diseases, such as heart diseases and cancer (Yazdanparast *et al.,* 2008). By measuring the conversion of the Fe^{3+}/ ferricyanide complex to the Fe^{2+}/ ferrous form, the antioxidant properties of the four extracts (acetone, ethanol, water and hexane) of *C. edulis* was examined and revealed that all of the solvent extracts and the reference drugs (butylated hydroxytuene and ascorbic acid) increased the reaction mixture's absorbance in a concentration-dependent manner (Park and Jhon, 2010). Even before compared to butylated hydroxytuene (BHT) and ascorbic acid, water extract had the greatest reducing power, accompanied by ethanol, acetone and hexane reported by Omoruyi *et.al.,* 2012. Water extract was comparable to the standard medications of BHT with rutin. Therefore, the outcomes demonstrated that, even at the minimum concentration (0.025 mg/ml), water and ethanol extracts have high 2,2-diphenyl-1-picryl-hydrazyl-hydrate (DPPH) scavenging activity (Omoruyi *et al.,* 2012). Only the ethanol extract had the largest percent inhibition at the lowest dosage response, making it superior to the conventional medications BHT and rutin while the aqueous extract had the lowest absorbance, followed by acetone and hexane stated by (Omoruyi *et al.,* 2012). This indicates that this plant extract may act as a free radical inhibitor even at low dose-response concentrations (Sultana *et al.,* 2009).

2.4.3.2. Antidiabetic properties of *C. edulis*

There has been evidence that certain phenolics have therapeutic effects for the managing of type-2 diabetes (Aryaeian *et al.,* 2017). Despite *C. edulis* well-known indigenous medicinal use in diabetes prevention and treatment (Malan *et al.,* 2006) and a study linking its extract's phenolic constituents to its antidiabetic ability (Mulaudzi *et.al.,* 2019). Over 90% of cases of diabetes are type 2, but the actual process or origin of own cause(s) among the specific enzymes connected to carbohydrate metabolism remains unknown (Sabiu *et al.,* 2021). Only water extract of the *C. edulis* leaves of the three solvents (water, 50 percent methanol, 70 percent acetone), tested against alpha-glucosidase fared comparably well in terms of activity (Mulaudzi *et al.,* 2019). This result confirmed the plant's ability to lower blood sugar levels and also supports the usage of polar solvents in herbal preparations or extraction methods in traditional Chinese medicine (Sabiu *et al.,* 2021). In comparison to the benchmarks acarbose but also ranirestat, the phenolic extract of *C. edulis* significantly ($p<0.05$) increased its antagonistic effects against alpha-glucosidase as well as aldose reductase, respectively, while favourably competing with acarbose for alpha amylase (Rehana *et al.,* 2017). All in all, the in vitro assay revealed that *C. edulis* leaves extracts strongly impeded -glucosidase enzymes (Mulaudzi *et al.,* 2019). A different study revealed that *C. edulis* fruit extracts in water, ethanol and acetone displayed low inhibition activity on the enzymes, alpha glucosidase and alpha amylase (Castañeda-Loaiza *et al.,* 2020).

2.4.3.3. Photoprotective properties of *C. edulis*

Bazzicalupo *et al.* (2021) reported that at a dose of 1000 µg/mL, the MTT assay showed that *C. edulis* only slightly induced cytotoxicity in HaCat keratinocytes, with an estimated IC_{50} > 1000 µg/mL. When compared to controls, the extract accelerated the wound - healing rate during the in-vitro tissue repair test. In comparison to the 100% wound closure induced by allantoin, the impact seemed to be dose-dependent and achieved up to 83% healing process. At five hundred and one thousand micrograms per millilitres CAE, in both, hyaluronidase showed inhibitory rates of 20% as well as 100%, with the latter being highly comparable to that of the control sample epigallocatechin gallate (EGCG). The wound-healing abilities of aqueous extract are confirmed by the data, which were gathered on an *in vitro* human skin model, avoiding the issues which use of an organic solvent elevates in skin care uses. Lassoued *et al.,* (2021) stated that, the phenolic composites, procyanidin B2, catechin, dihydromyricetin, as well as syringe-3-O-rutinoside remained the most prevalent constituents amongst twenty phenolic content recognised by LC-MS investigation and it is well known that catechin has anti-aging properties and can prevent and repair skin damage (Olayemi *et al.,* 2017). Syringetin-3-O-rutinoside, a flavanol that makes up 14,28% of the cumulative phenolic content of the *C. edulis* water extract, has demonstrated a protective effect on keratinocytes against reactive oxygen species and cells aging (Na *et al.,* 2003). At a highest concentration or a range about 80 to 90% UV (ultra-violet) blockage, *C. edulis* crude extract showed a significant photoprotective impact, according to the measured sun protection factor (SPF) values

(Imam *et al.,* 2015). A photoprotective effect of *C. edulis* aqueous leaf extract that is directly proportional to concentration is intriguing (Lassoued *et al.,* 2021).

2.4.3.4. Anticancer properties of *C. edulis*

Natural compounds known as bioactive phytochemicals are the secondary plant metabolites of medicinal herbs (Dadashpour *et al.,* 2011). Numerous studies have shown that phytochemicals are utilised as chemotherapeutic and preventative agents for different forms of cancer (Firouzai-Amandi *et al.,* 2022). The compounds isolated from *C. edulis* include; β-Amyrin (Kushiro *et al.,* 1998), Oleanolic acid (Agerbirk *et al.,* 2003), Uvaol (Mahato *et al.,* 1994 and Siddiqui *et al.,* 1986), monogalactosyldiacylglycerol (Sakano *et al.,* 2005), Catechin (Bilia *et al.,* 1996), Epicatechin (Chung *et al.,* 1997), Procyanidin B5 (Filipits, 2004). These isolated compounds exhibited a 50% inhibitory concentration (IC_{50}), suggesting an antiproliferative action against MDR1-transfected L5178 cells and parental (PAR) L5178 cells (Akinyede *et al.,* 2020). Each compound displayed a decreased the growth including both PAR and transfected MDR-1 cells. Moreover, uvaol and oleanolic acid had a greater impact on the parental cells, while MGDG and epicatechin had a greater impact on the MDR1-transfected cells. (Martins *et al.,* 2010).

The neuroblastoma cells, specifically, SK-N-BE(2) and SH-SY5Y, were more susceptible to *C. edulis* leaf water extract than control cells, resulting in dose-dependent cytotoxicity hence implying that SH-SY5Y cells were less responsive to treatment than SK-N-BE(2) cells (Ekpo *et al.,* 2021). At concentrations that block the T cell lymphoma cells L5178 of the mouse's verapamil-sensitive efflux pump, the extract is non-toxic, making the previously drug-resistant cells susceptible to new anticancer medications (Ordway *et al.,* 2003). Using the HCT-116 malignant cells lines as a test subject, the water-ethanol and aqueous extract of the *C. edulis* both were found to inhibit cell proliferation as measured by the MTT and after 24 hours of treatments, significant *P-value* of less than 0.05 and 0.01 differences, respectively, in aqueous and ethanol-water extract were discovered in comparison to the negative control (Hafsa *et al.,* 2016).

2.4.3.5. Antimicrobial properties of *C. edulis*

Studies on plant antimicrobial properties have progressed from interdisciplinary experiments, where researchers employed antimicrobial agents' information to support the phytoconstituents research, to further specialized investigations regarding particular data about the plants' antimicrobial properties (Van Vuuren, 2008). The phytochemicals had already demonstrated significant action against a wide range of microbial species (Mudimba *et al.,* 2019). The phytochemical constituents extracted from ethyl-acetate and semi-purified tannin compounds, specifically, rutin, neohesperidin, hyperoside, cactichin, ferulic acid, and flavonoids, exhibited significant antibacterial properties against the gram-negative, *Moraxella catharalis*, the gram-positive cocci, *Staphylococcus aureus,* and *Staphylococcus epidermidis* (Van Der Watt *et al.,* 2001). The ethanolic extract of *C. edulis* remained shown to be significantly efficient with *Staphylococcus aureus* and *Bacillus cereus*, although it showed little action against *Escherichia coli* and *Klebsiella pneumoneae* (Buwa *et al.,* 2009). In three tested bacterial strains, *Staphylococcus aureus* (MIC 0.4%), *Pseudomonas aeruginosa* and *Escherchia coli*, *C. edulis extracts* demonstrated minimal inhibitory concentrations (MIC), which indicated a significant antibacterial property (Ibtissem *et al.,* 2012). Based on the crude extract, *Carpobrotus edulis* extract exhibited superior bactericidal effect against *Escherichia coli* (MBC 50%) and similar slight bactericidal concentrations (MBC) against *Staphylococcus aureus* (MBC 100%) and *Pseudomonas aeruginosa* (MBC 100%) reported by Ibtissem *et al.,* 2012. With minimal inhibitory concentration, these essential oil extracts shown antifungal efficacy on *Candida* species (*krusei, albican, glabrata, rugosa*) and *Cryptococcus neoformans* (Omoruyi *et al.,* 2014). All five of the fungal isolates were susceptible to hexane extracts; however, acetone extracts have only been active toward *Candida krusei* (Mudimba *et al.,* 2019).

2.4.3.6. Anti-inflammatory properties of *C. edulis*

Swelling is one of the body's initial biological and immunological reaction and serves as a defence mechanism against stimuli that might be detrimental, such as metabolic stresses, injuries, or infections (Carro *et al.,* 2015). Inflammatory reactions to chemicals found in plants, such as phytoestrogens, flavonoids, and their metabolites, can be inhibited by these molecules (Iwalewa *et al.,* 2007). Several inflammatory mechanisms exist, including cyclooxygenase (COX) and lipoxygenase (LOX) which both of the prostaglandin synthases that catalyse the successive production for prostaglandin G2 (PGG2) and prostaglandin H2 (PGH2) using arachidonic acid (AA) by means of intrinsic COX and peroxidase actions (Rajan *et.al.,* 2016). Many anti-inflammatory substances, including flavonoids, phenols, tannins, and curcumins, are hypothesized to work by scavenging free radicals or by inhibiting COX and LOX which are pro-inflammatory enzymes (Ondua *et al.,* 2019). Nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) and Mitogen-activated protein kinases (MAPKs) are crucial players in producing pro-inflammatory mediators, which are two examples of significant signalling pathways that bioactive compounds and their constituent molecules may inhibit to exhibit their biological effects (Arulselvan *et al.,* 2016). The *C. edulis* acetone extracts had very high levels of nitric oxide (NO) generation inhibition of 97.88% (Ondua *et al.,* 2019). Leukotrienes are messengers of a variety of pro-inflammatory substances, and aiming at 15-LOX is regarded as a potential beneficial approach in the therapy of inflammatory syndromes (Schneider *et al.,* 2005). Potent anti-inflammatory medications may be produced from plant extracts or chemicals that stop the lipoxygenase enzyme's pro-inflammatory actions (Adebayo *et al.,* 2015). Inflammatory molecular targets including 15-LOX enzymes, reactive oxygen species (ROS), and reactive nitrogen species (RNS) pathways have been implicated as secondary bioactive compounds by organic products with significant phenolic contents potentially limit the inflammatory response (Mittal *et al.,* 2014).

2.4.3.7. *C. edulis* effects on chromium VI

With chromium (III) and chromium (VI) as the most abundant and widespread oxidation forms oof chromium (Cr), chromium comprises one of the primary production wastes that causes health difficulties in both animals and human beings (DesMarias *et al.,* 2019). Compared to chromium (III), Cr (VI) seems to be more hazardous and readily absorbed, remarkably when consumed by mouth and penetrating the cell through assisted diffusion (Karaulov *et al.,* 2019). As stated in the literature, lung cancer is most frequent in industrial employees whom are predisposed to Cr (VI) via inhalation whereas it has been discovered that ingesting Cr (VI) from drinking water causes cancer (Sun *et al.,* 2015).

Heavy metal ions are said to immediately influence the haemopoietic stem cell lines within spleen and kidney with unexpected membrane fluidity, triggering iron deficiency anaemia through lowering the supply of oxygen as a result of the lower erythrocytes concentration and haemoglobin concentrations (Kumar *et al.,* 2016). However, the treatment with *C. edulis* leaf pulp increased the concentrations of haemoglobin (Hb), polycythaemia (PCV), red blood cells (RBC), mean corpuscular haemoglobin concentration (MCHC) and mean corpuscular haemoglobin (MCH), noticeably, implying that the plant may be able to fend off the harmful effects of chromate (Obaseki *et al.,* 2021). White blood cells in Group 4 groups that received body mass of Cr (VI) five milligram per kilogram alone displayed a statistically importance P-value which is less than zero comma zero five increment in comparison to control, that is consistent with the data (Suljević *et al.,* 2021). The anticancer feature of *C. edulis* extract, which may have reduced the degree of generation of undeveloped white blood cells, may have caused overall white blood cell count to normalize in the study's treated groups associated to the monitor group (Omoruyi *et al.,* 2019). One of the fundamental biomarkers of kidney damage such as in chromium-treated animals seems to be an increase in plasma urea (Obaseki *et al.,* 2021). This raised amount was noticed in treated groups with Cr (VI), revealing renal disfunction; consequently, in the treated groups with *C. edulis extract*, a huge reduction in urea amount was seen, denoting that the extract improved the kidney (Obaseki *et al.,* 2021). The study's findings indicate that now *C. edulis* leaf pulp decreased the toxicity brought on via Cr (VI) and It remained found that the decrease was larger in therapy groups compared to the pre-treatment groups (Obaseki *et al.,* 2021). The study is targeted at this particular type of medicinal plant of interest (*C. edulis*), as it has not been investigated against cervical cancer.

3. Introduction

This chapter outlines every tool and technique employed during the study to accomplish the aim and objectives of this research project. All experiments that were carried out more than once will only be stated once to prevent duplication, but any modifications that were made will be appropriately noted. The following methods were used; Analysis of phytochemicals for both qualitative and quantitative of *C. edulis* extracts, MTT assay, apoptosis and human proteome profiler targeting angiogenesis related proteins.

3.1. Materials

3.1.1. Plant collection and verification

Carpobrotus edulis plant was harvested in the Limpopo Province, near Ga-Mamabolo, Segoreng village. A specimen voucher number (UNIN 1220092) was assigned when specialists at the University of Limpopo's Larry Leach Herbarium confirmed the plant's authenticity.

3.1.2. Plant extraction

The leaves and roots of the plant were rinsed with running tap water, air dried and crushed to a fine powder with an electric blender. The extraction solvents used were acetone, ethanol and distilled water as described by (Laka *et al.,* 2021). The extraction samples were air-dried after being filtered into glass beaker that had been previously weighed. For phytochemical analysis, the dried *C. edulis* extracts were re-dissolved within the corresponding solvents used for extraction while for all cell culture-based assays, they were reconstituted in dimethyl sulphoxide (DMSO) [Sarchem, RSA] for both acetone and ethanol extracts while for water extracts was re-dissolved in distilled water.

3.1.3. Chemicals and consumables

Iron(III)chloride, lead(IV) acetate, chloroform, sulphuric acid, hydrochloric acid, acetic acid, sodium hydroxide, benzene, ammonia, isoamyl alcohol, iodine, disodium hydrogen phosphate, sodium dihydrogen phosphate, ferricyanide potassium, trichloro acetic acid, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), 2,2-diphenyl-1-picrylhydrazyl (DPPH), methanol, vanillin, p-anisaldehyde, curcumin, ethyl acetate, formic acid, toluene, ethanol and ammonium hydroxide where purchased from Sigma-Aldrich (South-Africa).

3.1.4. Cell lines

Noncancerous [Hek-293 (ATCC® CRL-1573 ™)], cervical cancer cell lines [CaSki (ATCC® CRL-1550 ™)] and [HeLa (ATCC® CCL-2 ™)] purchased from American Type Culture Collection (ATCC, Manassas, USA) were employed as *in vitro* models in this research project. All the cell lines were cultivated under biosafety level 2 cabinet in accordance with manufacturer's procedure and were kept in cultivation flasks at 37°C in a saturated incubator comprising of 5% carbon dioxide.

3.1.5. The human angiogenesis protein profiler array kit

In order to characterize angiogenesis associated proteins in human cervical cancer cells, a Human Proteome Profiler Angiogenesis Antibody Array (Catalog # ARY007) [R&D Systems, Inc. (USA)] was used in the study. By measuring the mean spot pixel concentrations from each membrane array by means of picture computer software [G:box Chemi XX9 scanner (Syngene, India)], histogram profiles for specific analytes were produced.

3.2. Methods

3.2.1. Qualitative analysis of phytoconstituents of *Carpobrotus edulis* extracts

The following phytoconstituents were tested for in various extracts: leucoanthocyanins, flavonoids, saponins, tannins, glycosides, coumarins, phenols and carbohydrates. Leucoanthocyanins was detected by adding 5 mL of acetone, ethanol, and water to 5 mL of isoamyl alcohol, which was then mixed with *C. edulis* leaf and root extracts. The presence of leucoanthocyanins is evidenced by the upper layer showing reddish. 0.5 mL of less concentrated *C. edulis* leaves and roots extract in isopropyl alcohol was mixed with 1 mL of 100% ethanol and 3 drops of concentrated hydrochloric acid to determine the presence of flavonoids. The development of magenta, crimson, or orange coloration suggested the presence of flavonoids. To determine if saponins were present, 10 drops from *C. edulis* extracts diluted in 20 mg/mL isopropyl alcohol were added to 1 mL of the distilled water in a test tube, shaken rapidly to create froth, and then let rest for 10 minutes. The levels of saponin were assessed as follows: 6 mm high (moderate); 8 mm high (rich); no foam (absent); and a value lower than 3 mm height (poor). To find tannins, 10 mg of each *C. edulis* extracts were diluted in 1 mL of ethanol, followed by the addition of 2 mL of distilled water and 4 drops of a 10% w/v ferric chloride aqueous solution. The development of a green or blue colour suggested the existence of glycosides by adding 1 mL of *C. edulis* extracts, 1.5 mL of glacial acetic acid, a single drop of 5% ferric chloride, and concentrated H_2SO_4, glycosides were found. A presence of glycosides was shown by a blue-coloured solution. The presence of coumarins was determined by putting 0.5 g of *C. edulis* extracts to a test tube, covering the test tube's mouth with filter paper coated with 1N NaOH, and heating it in a water bath for a short while. The presence of coumarins was shown by the yellow fluorescence on paper when exposed to UV light. By adding a few drops of a 5% ferric chloride mixture to *C. edulis* extracts, phenols were found. The colour was dark green/bluish black, indicating an abundance of phenols. By adding 5 mL of 5% KOH solution to *C. edulis* extracts, carbohydrates were found. Carbohydrates were denoted by a yellow colouration.

3.2.2. Plant extracts profiling fingerprint using thin-layer chromatography (TLC)

Each of the types of chromatography works on the similar idea. All of them consists of a stationary phase (a gas or a liquid supported by a solid) and a phase which is mobile (a liquid or a solid). The mixture dissolves in a solution called the phase of mobility, which moves it through a structure that contains a distinct material referred to as the phase that is stationary. The mobile phase carries the contents of the mixture's component elements through the phase of stationary motion. The blend splits as a result of the varied speeds at which its constituents move. The separation is based on distinct ionization between both stationary and mobile stages (Tiwari and Talreja, 2022).

All the plants extracts were redissolved in different solvents (acetone, ethanol and water) utilized initially for extraction of phytoconstituents of the *C. edulis* leaves, roots and the juice extract to the concentration of 10mg/ml. For each extract, 20µl of 10 mg/ml spots were placed on aluminium TLC plates (Sigma-Aldrich, Germany) and then dipped into various solvents with different polarity, including Chloroform/Ethyl acetate/Formic acid (CEF); 10:8:2, Ethyl-acetate/Methanol/Water (EMW); 10:1.35:1, and Toluene/Ethanol/Ammonium hydroxide (TEA); 18:2:0.2, in saturated chambers (Kudumela *et al.,* 2018). The first three plates in each set of three TLC plates were exposed to TEA, EMW, and CEF. In order to develop the colour, they were heated to 105 ^{0}C degrees and sprinkled using prepared vanillin (1ml of p-anisaldehyde, 18ml of ethanol, 1ml of sulphuric acid and 0.1g of vanillin) and p-anisaldehyde (Kudumela *et al.,* 2018). The second set of three plates TLC plates were dipped into TEA, EMW, CEF chambers respectively, and sprinkled using 0.2 % of 2,2-diphenyl-2-picryl-hyrazyl (DPPH) from (Sigma-Aldrich, Germany) for detection of antioxidants in various plant crude extracts. The yellow stains were identified against purple overlay on sprayed TLC plates with 0.2 % DPPH dissolved in methanol.

3.2.3. Quantitative test for the scavenging of free radicals by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)

The free radical assay known as 2,2-diphenyl-1-picryl-hydrazyl (DPPH) is an antioxidant assay that electrons are transferred to create a violet-coloured mixture in ethanol. When an antioxidant molecule is present at room temperature, this free radical is steady which reduces an ethanol solution without colour results. DPPH is diminished when it interacts with an antioxidant that has the ability to donate hydrogen. The hue shift (from pale yellow to deep violet) [Garcia *et al.,* 2012]. Activities that scavenge free radicals of the *C. edulis* (acetone, ethanol, water and juice) extracts of both leaves and roots were measured and compared utilising previously published methodology for the DPPH test modified in certain ways (Laka *et al.,* 2021). At varying concentrations at (15.63, 31.25, 62.50, 125, 250, 500, and 1000 µg/ml), 250µl distinct *C. edulis* extracts both for leaves and roots were made, and 500µl DPPH of 60µg/ml (Sigma-Aldrich, USA) reagent were added. Followed by incubation period of 30 minutes at 25^{o}C in a dark area whereby ascorbic acid served as a positive control. The samples' absorbance readings at 517 nm were obtained utilizing microtiter plate analyser (Promega, USA) following the incubation period, and the % inhibition was calculated using the formula (1).

$$\%Scavenging\ activity = \frac{blank\ absorbance\ 517 - sample\ absorbance\ 517}{blank\ absorbance\ 517}\ x\ 100 \qquad (1)$$

3.2.4. Quantitative test for total antioxidants capacity by ferric reducing antioxidant power (FRAP)

The ferric reducing antioxidant power (FRAP) assay relies on a principle that at low pH, a sample's antioxidants reduce the ferric-tripyridyl triazine ($Fe3^{+}$-TPTZ) complex to ferrous tripyridyl triazine ($Fe2^{+}$-TPTZ). Ferrous tripyridyl triazine ($Fe2^{+}$-TPTZ), is blue in colour, and the antioxidant capability is associated with change in absorbance (Benzie and Devaki, 2018). An antioxidant power of ferric reducing ions (FRAP) of the *C. edulis* (acetone, ethanol, water and juice) extracts of both leaves and roots was determined using the previously outlined procedure (Kudumela *et al.,* 2018). At various concentrations at (15.63, 31.25, 62.50, 125, 250, 500, and 1000 µg/ml), *C. edulis* extracts of 500µl for both leaves and roots were added in 15ml centrifuge tubes. Followed by an addition of 500µl 0.2M sodium phosphate buffer and 500µl of 1% potassium ferricyanide were vortexed thoroughly. The concoctions were kept at the temperature of 50°C for 20 minutes. After incubation, 10% of trichloroacetic acid was added and the samples were centrifuged for about 30 minutes at 3000 rpm. In a fresh 15 ml centrifuge tube, 1ml of the supernatant was added followed by addition of 5ml of distilled water, then 0.1% ferric chloride of 1ml was added. Absorbances were measured at 700 nm using a microtiter plate reader with ascorbic acid as the positive control (Promega, USA). The percentage of inhibition was calculated using the formula (2) shown below.

$$\%scavenging\ activity = \frac{blank\ absorbance\ 517 - sample\ absorbance\ 517}{blank\ absorbance\ 517}\ x\ 100 \qquad (2)$$

3.2.5. Ultra-high performance liquid chromatography-Ultra-violet/Diode array detector (UHPLC-UV/DAD) Analysis

A Phenomenex Luna C18 column of this particle size (100 × 2 mm, 2.5 µm) was used to achieve the separation and the components of the system were Sciex ExionLC-UV/DAD (Framingham, USA). Zero comma one percent formic acid was utilised as the mobile phase in both A (water) and B (methanol). 0 to1 minute (5% B), 1 to 22 minutes (95% B), 22 to 27 minutes (95% B), 27-27 minutes and 10 seconds (5% B), and 27 minutes 10 seconds to 30 minutes (5% B) were the gradient elution times. Each one of the samples of *C. edulis* (acetone, ethanol and water extract) had a duration run of 30 minutes and a flow rate of 0.4 milliliters per minute. For analysis, 10µL of every sample were put into the apparatus. At 40°C, the column oven was adjusted. The wavelength at which the UV detector was obtained was 254 nm.

3.2.6. Ultra-high performance liquid chromatography-Mass spectrophotometry (UHPLC-MS) Analysis

Sciex ExionLC system coupled to Sciex X500R QTOF system containing electron spray ionisation (ESI) probe were used for analysis. The chromatographic conditions and liquid chromatography (LC) column employed were identical to those documented for the UHPLC-UV/DAD system (Framingham, USA). N2 was the desolation gas and the mass spectrometer was running in positive ion electrospray mode. Ion source of a temperature 500°C, spray voltage of 5500, de-clustering potential at 50 Voltage, ion source gases 1 and 2 at 50 psi and 70 pressure, separately, curtain gas at 30 psi, and collisional activated dissociation (CAD) gas at 7 psi. Analytic software was employed for both gathering and handling data at the Department of Chemistry, University of Limpopo.

3.2.7. Cell culture maintenance and treatment

In a humidified chamber containing 5% CO_2, the HeLa (cervical cancer cell line) and Hek-293 cells (non-cancerous) were grown in 37°C in a Dulbecco's Modified Eagle's Medium (DMEM) and the CaSki cells in a Roswell Park Memorial Institute (RPMI) 1640 medium enriched with 10% foetal bovine serum (FBS) and 1% Penicillin-Streptomycin-Neomycin (PSN). The cultivated cells remained maintained as per manufacturer's protocol (ATCC, USA) by replacing their old growth media with new one after twice being rinsed with two millilitres of 1X phosphate buffered saline (PBS). A minimum of once a week, after they reach 80-90 percent confluency; the cell lines were sub-cultivated, and any extra subcultures were stored in a freezer of -80°C in DMEM growth media enhanced with 5% dimethyl sulfoxide (DMSO) and 10% FBS.

In order to achieve the final concentrations needed for the treatment of Hek-293, HeLa, and CaSki cells, *C. edulis* extracts (acetone, ethanol, and water) at a (5×10^4) µg/ml and a (3×10^2) µM stock solution of curcumin were diluted in the whole medium. All cell lines were set up at a density of the following cells/ml (1×10^5) and cultured at the temperature of 37°C for a duration of the experimental setup. Following that, the cells were exposed to doses of *C. edulis* extract (15.63-1000 µg/ml) in acetone, ethanol, and water for 24 hours. As a positive control, curcumin was employed.

3.2.8. Cytotoxicity and cell viability analysis

The MTT assay is one of the colorimetric techniques employed to evaluate cellular growth and cytotoxicity. Under some circumstances, the response of cellular oxidoreductase enzymes dependent on nicotinamide adenine dinucleotide phosphate (NADPH) may indicate quantity of live cells in the environment. When solubilized for spectrophotometer measurement, the tetrazolium dye MTT can be reduced by these enzymes because of its impermeable formazan form that is purple-coloured. The *C. edulis* acetone, ethanol and water extracts were evaluated for their cytotoxicity and cell viability against Hek-293, HeLa, and CaSki cells using MTT test.

The MTT test was then utilised to evaluate an impact of several *C. edulis* isolates and the positive control, curcumin, on cell viability of Hek-293, CaSki, and HeLa cells. Briefly, HeLa and CaSki cervical cancer cell lines, as well as non-cancerous Hek-293 cells, were seeded at a concentration of 1 × 10^5 cells per well in a 96 well microtiter plate. Varying concentrations of *C. edulis* extracts (15.63-1000 µg/ml) and curcumin (0-60 µM) were added and the cells were gestated for 24 hours after being allowed to attach for the night. After 24hr incubation, media was removed from the plates and 10 µl of 5 mg/ml MTT reagent was added into each and every well, and the plates were then incubated for an additional four hours at 37°C. Followed by the aspiration of the MTT solution, a DMSO of 100µl was supplemented to each well to facilitate the dissolution of the formazan crystal generated by live cells. This allowed for the measurement of absorbance at 560 nm by means of a microtiter plate analyser (Promega, USA). The following formula (3) was applied to get the percentage of cell viability.

$$Cell\ viability\ (\%) = \frac{sample\ absorbance\ 560nm}{untreated\ control\ absorbance\ 560nm}\ x\ 100 \qquad (3)$$

The viability of the cells was assessed with Muse™ count and viability kit from (Merck Millipore, Germany) in accordance with the manufacturer's directions in order for further validation of the MTT test. Following exposure to various treatments, adherent cells were washed with 250µl per well of PBS then detached with 100µl 1X trypsin and floating cells were recovered by centrifugation. The trypsin was inactivated by adding full media, which was then centrifugated at 300 relative centrifugal force (RCF) for a period 5 minutes. The pelleted cells were then re-suspended in a fresh complete media of 20µl, combined by a volume of 100 µL with Guava Via-Count mixture, and incubated for five minutes in a dark area. Next, all the samples were examined with Merck-Millipore's Muse® Cell Analyzer (Germany).

3.2.9. Apoptosis analysis

The Annexin V & Dead Cell Kit of Muse™ (Merck Millipore, Germany) was employed in accordance with the manufacturer's guidelines to ascertain how IC_{50} of *C. edulis* water extract (WE) affected the cell viability of HeLa and CaSki cells as assessed by the cell viability tests. Briefly, the HeLa and CaSki cells were cultivated in 6-well plates at a concentration of 1 × 10^5 cells per well for 24 hours in DMEM and RPMI1640 media supplemented with 10% FBS, respectively. After 12 hours of synchronization, cells were then treated for a period of 24 hours with 40 µM and 50 µM curcumin, respectively, and 72 hours with 1000 µg/ml *C. edulis* (WE). After being subjected to various treatments of IC_{50}, suspended cells were separated by centrifugation, and adherent cells were added trypsin and cleansed with PBS of 1X concentration. To deactivate the trypsin, the complete media with FBS was added. Following the transfer of the cell suspension of 100µl to Eppendorf tubes, 100 µl of Muse® apoptotic cocktail was added, and the samples were left to incubate in a dark area for a period of 20 minutes. Using the Muse® Cell Analyzer (Merck-Millipore, Germany), the proportion of apoptotic cells following treatment with *C. edulis* (WE) and curcumin was measured and examined.

3.2.10. Protein extraction

ThermoFisher Scientific (USA) supplied the radioimmunoprecipitation assay (RIPA) lysis buffer that was used. The proteins were extracted from HeLa cells in accordance with the manufacturer's instructions. All in all, the HeLa cells were then cultured in 6-well culture plates for 24 hours at a concentration of 1×10^5 cells per well. 40 µM curcumin and 1000 µg/ml *C. edulis* (WE) were applied to the cells during 24 and 72 hours, respectively. Adherent cells were detached using 1X trypsin after being exposed to several treatments and twice washed with cold 1X PBS. Cells which were suspended were then sorted by centrifugation at 12000 x *g* for a period of 5 minutes. After the pellet was put on ice and vortexed for 30 seconds, 250µl of RIPA lysis buffer was added. The solution was put on ice and vigorously shaken for half an hour on a shaker. The samples were moved to fresh Eppendorf tubes that were set on ice for further quantification after being centrifugated at 12000 x *g* for a period of 30 minutes at the temperature of 4^oC.

Quantification of protein samples was done using Bradford protein assay (BSA) purchased from ThermoFisher Scientific, USA. The Bradford test is a rapid and relatively sensitive way to quantity the amount of protein available. The method of the maximum absorbance of the Coomassie Brilliant Blue G-250 dye shifts from 465-595nm after coupling to proteins that have denatured in a mixture. In accordance with the manufacturer's instructions, reagents A and B were combined in a 50:1 ratio to make a functional BCA (Bicinchoninic acid) reagent. Then, 200µl of BCA was added 25µl of the samples. After incubating the samples with BCA mixture for a period of 30 minutes at 37^oC, the absorbance was read at the wavelength of 560 nm.

3.2.11. Determination of the levels of human angiogenesis-related proteins

Understanding the functions of angiogenesis related proteins performed in mechanisms pertaining to the physiological process of creating new blood vessels was made easier by analysing their expression patterns according to manufacturer's protocol (R&D Systems, Inc, USA). Without having to run separate tests for each protein, the human angiogenesis array kit offers a quick and affordable way to find the comparative levels of expression for 55 angiogenesis related proteins at the same time. On nitrocellulose membranes, properly chosen capture antibodies were actually spotted in duplicate. Samples were combined with a variety of biotinylated detecting antibodies after being diluted. The human angiogenesis array was then treated with the sample/antibody combination. Any protein/detection antibody combination that was present was bound to the membrane by its corresponding immobilized capture antibody. Chemiluminescent detection reagents and streptavidin-HRP were added in order after a wash to remove unattached particles. Each spot generated light in accordance to the bound analyte quantity. Each nitrocellulose membrane was analysed using ImageJ software (LOCI, University of Wisconsin).

3.2.12. Statistical analysis

All experiments will be done in triplicates in three independent repeats. Graph Pad Prism 8.0 and Graph Pad Instat 3.06 software will be used to analyse the data. Analysis of variance (ANOVA) will be used to make multiple comparisons and data will be presented as mean + Standard Deviation. Statistical significance between two sets of data will be defined as a p-value of less than 0.05 and comparison of each sample will be done with the control of untreated cells.

4. Introduction

According to published research, a select few medicinal plants, such as *C. edulis*, have medicinal properties for the treatment of a wide variety of illnesses (Thring and Weitz, 2006). The well-established practice of using herbal remedies in developing new drugs calls for careful investigation of a number of factors, such as the plant's traditional use by the inhabitants of the region, its geographic distribution and accessibility in significant amounts, and its long-term natural longevity (Akinyede *et al.,* 2020). Despite the fact that *C. edulis* is one of the *Carpobrotus* species that fits these requirements, its additional applications seem to outweigh its medical and nutritional benefits. Because there is a paucity of published data on *C. edulis* against cervical cancer, our goal was to assess the extracts' possible anticancer efficacy against several cervical cancer cell lines. To achieve these objectives: qualitative and quantitative analysis, MTT assay, flow cytometry and human proteome profiler techniques were used.

4.1. Qualitative analysis of phytoconstituents of *Carpobrotus edulis* extracts

The following phytoconstituents were tested for in various extracts: leucoanthocyanins, flavonoids, saponins, tannins, glycosides, coumarins, phenols and carbohydrates. Leucoanthocyanins was detected by adding 5 mL of acetone, ethanol, and water to 5 mL of isoamyl alcohol, which was then mixed with *C. edulis* leaf and root extracts. The presence of leucoanthocyanins is evidenced by the upper layer showing reddish. 0.5 mL of less concentrated *C. edulis* leaves and roots extract in isopropyl alcohol was mixed with 1 mL of 100% ethanol and 3 drops of concentrated hydrochloric acid to determine the presence of flavonoids. The development of magenta, crimson, or orange coloration suggested the presence of flavonoids. To determine if saponins were present, 10 drops from *C. edulis* extracts diluted in 20 mg/mL isopropyl alcohol were added to 1 mL of the distilled water in a test tube, shaken rapidly to create froth, and then let rest for 10 minutes. The levels of saponin were assessed as follows: 6 mm high (moderate); 8 mm high (rich); no foam (absent); and a value lower than 3 mm height (poor). To find tannins, 10 mg of each *C. edulis* extracts were diluted in 1 mL of ethanol, followed by the addition of 2 mL of distilled water and 4 drops of a 10% w/v ferric chloride aqueous solution. The development of a green or blue colour suggested the existence of glycosides by adding 1 mL of *C. edulis* extracts, 1.5 mL of glacial acetic acid, a single drop of 5% ferric chloride, and concentrated H_2SO_4, glycosides were found. A presence of glycosides was shown by a blue-coloured solution. The presence of coumarins was determined by putting 0.5 g of *C. edulis* extracts to a test tube, covering the test tube's mouth with filter paper coated with 1N NaOH, and heating it in a water bath for a short while. The presence of coumarins was shown by the yellow fluorescence on paper when exposed to UV light. By adding a few drops of a 5% ferric chloride mixture to *C. edulis* extracts, phenols were found. The colour was dark green/bluish black, indicating an abundance of phenols. By adding 5 mL of 5% KOH solution to *C. edulis* extracts, carbohydrates were found. Carbohydrates were denoted by a yellow colouration (Shaikh & Patil, 2020).

Table 1: Test for the presence/absence of phytoconstituents using biochemical tests.

		Leaves			**Roots**		
Phytoconstituents	Juice extract	Acetone extract	Ethanol extract	Water extract	Acetone extract	Ethanol extract	Water extract
Tannins	+	++	+	-	+++	++	-
Flavonoids	+	+	-	++	-	-	++
Phenols	-	++	++	-	++	++	-
Saponins	-	-	-	-	+	+	-
Steroids	+	++	++	-	+++	+++	-
Glycosides	-	-	-	-	-	-	-
Coumarins	+	++	+++	+	+++	++	+
Proteins	-	+	-	-	-	+	-
Leucoanthocyanins turns	-	-	-	-	-	-	-
Carbohydrates	+	+	++	-	++	+	-

Key: (-) Absent, (+) Present, (++) Moderately present, (+++) Strongly present

The presence of various phytochemical groups, such as tannins, flavonoids, phenols, saponins, steroids, coumarins, proteins, leucoanthocyanins turns and carbohydrates in *C. edulis* extracts showed in Table 1 were analysed by assessing the colour change. In comparison to water extracts, both acetone and ethanol extracts from the leaves and roots contain the most of the phytochemical groups. Root and leaf water extracts were found to contain coumarins and flavonoids. The juice extract included tannins, flavonoids, steroids, coumarins, and carbohydrates. This class of phytochemicals is widely recognized for its possible antioxidant properties, anticancer activities, antibacterial, and anti-inflammatory therapeutic properties (Laka *et al.,* 2021; Panche *et al.,* 2016). Carbohydrates were found in the juice extract, acetone, and ethanol (for both the leaves and the roots), but proteins were only found in the acetone and ethanol extracts of the leaves and the roots, respectively.

4.2. Plant extracts profiling fingerprint using thin-layer chromatography (TLC)

In order to achieve a concentration of 10 mg/ml for the phytoconstituents of the *C. edulis* leaves, roots, and juice extract, all of the plant extracts were redissolved in the three separate solvents (acetone, ethanol, and water) that were used at the time of the first phytoconstituent extraction. Chromatograms produced following the development of plant extracts in the TEA, CEF, and EMW mobile phases and sprayed with ready-to-use p-anisaldehyde and vanillin (1ml p-anisaldehyde, 18ml ethanol, 1ml sulphuric acid, and 0.1g vanillin) in **figures 1 – 3**.

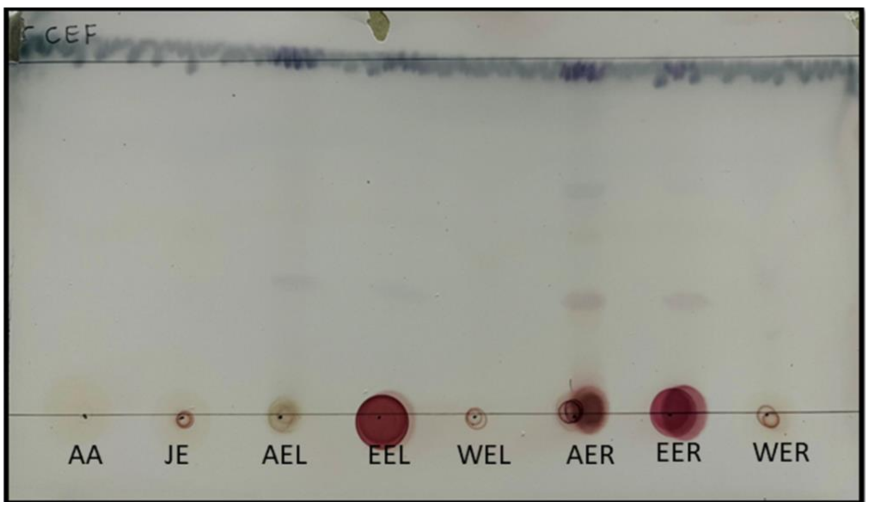

Figure 1: TLC plate CEF

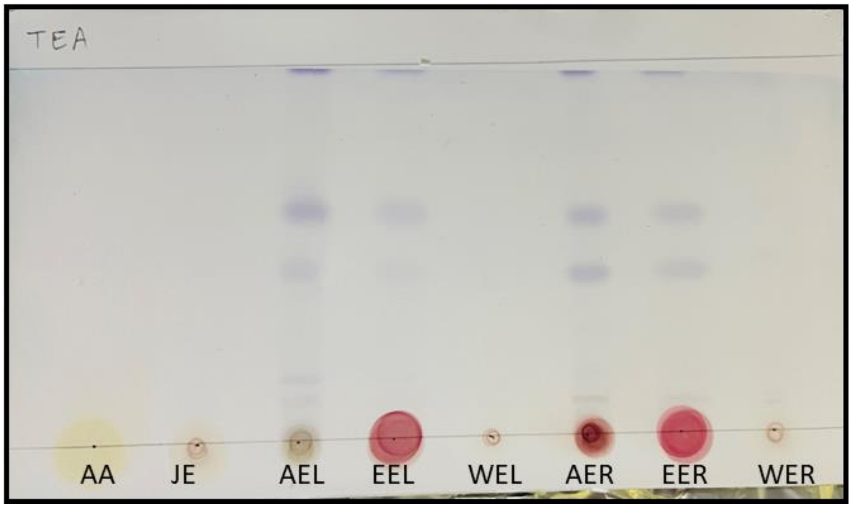

Figure 2: TLC plate TEA

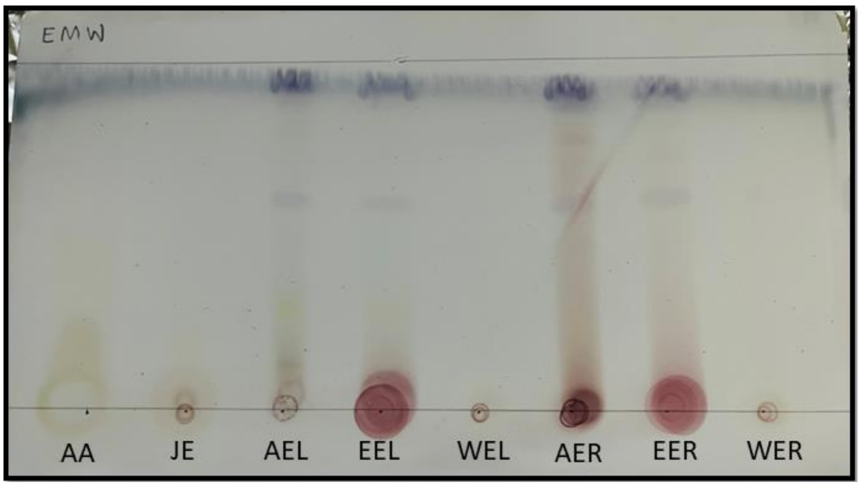

Figure 3: TLC plate EMW

Abbreviations: AA (Ascorbic Acid), JE (Juice Extract), AEL (Acetone Extract Leaves), EEL (Ethanol Extract Leaves), WEL (Water Extract Leaves), AER (Acetone Extract Roots), EER (Ethanol Extract Leaves), WER (Water Extract Roots).

The following three-plate set for identifying antioxidants in various plant crude extracts, TLC plates were dipped into TEA, EMW, and CEF chambers, respectively, and sprayed with 0.2% 2,2-diphenyl-2-picryl-hyrazyl (DPPH) [Sigma-Aldrich, Germany]. When TLC plates sprayed with 0.2% DPPH in methanol, the yellow stains were visible against a purple background which indicated antioxidant properties in figure 4-6.

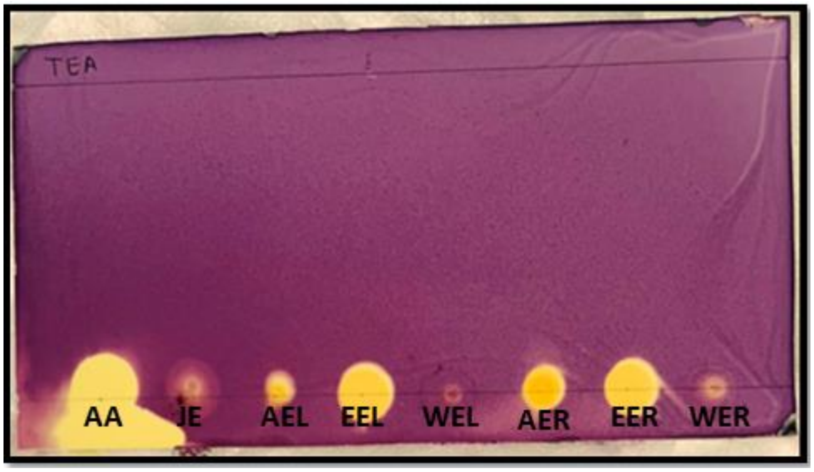

Figure 4: TLC plate TEA DPPH

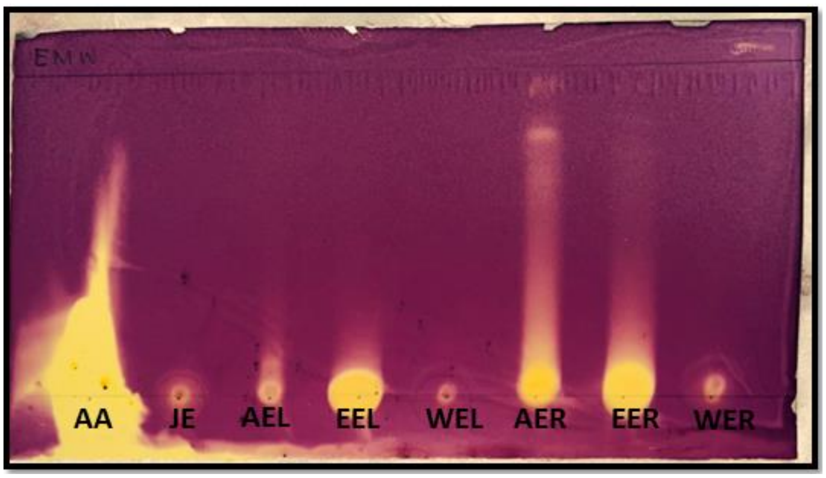

Figure 5: TLC plate EMW DPPH

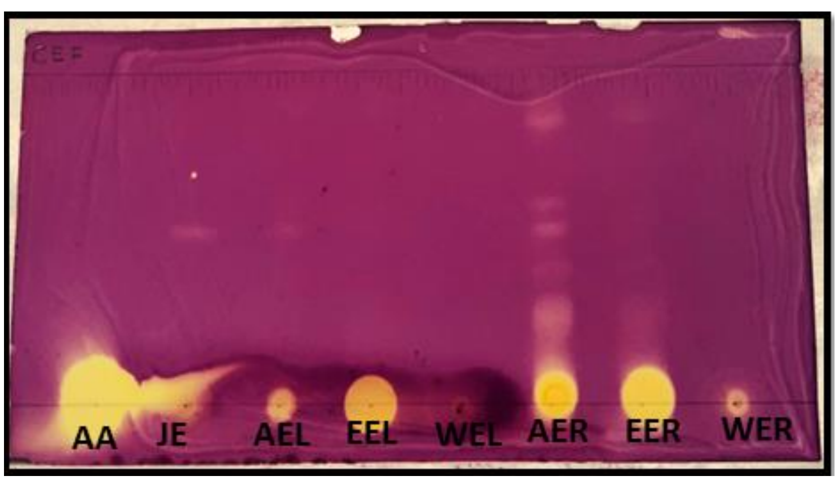

Figure 6: TLC plate CEF DPPH

Abbreviations: AA (Ascorbic Acid), JE (Juice Extract), AEL (Acetone Extract Leaves), EEL (Ethanol Extract Leaves), WEL (Water Extract Leaves), AER (Acetone Extract Roots), EER (Ethanol Extract Leaves), WER (Water Extract Roots).

4.3. Quantitative test by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) of free radicals

A relationship between antioxidant properties in some medicinal herbs has been observed in earlier studies (Luo *et al.,* 2004; Sun *et al.,* 2015). There was a significant difference in the amounts of the three solvents' extracted components from the crude extract, indicating that each solvent has a distinct capability for extracting polyphenols (Omoruyi *et al.,* 2012). The vivid purple colour of the DPPH free radicals is caused by an unusual electron in these molecules (Mohamed *et al.,* 2009). When an antioxidant molecule donates an electron to DPPH, the resultant decolourisation can be quantified by changes in absorbance (Wei *et al.,* 2010). This was shown in our experiment as soon as the colour changed to purple towards yellow, showing that the decreased DPPH-H at 517 nm is formed when the unusual electron of the DPPH radical is linked with hydrogen from an antioxidant that scavenges free radicals. In **table 2 and figure 7**, the maximum DPPH inhibition was seen at 1000 µg/ml in ethanol and acetone extract of the leaves, with values of 65.337 ± 1.208 and 53.320 ± 3.381, respectively. Leaf water extract had a value of (39.383 ± 1.035) at a concentration of 1000 µg/ml as equated to ascorbic acid (86.817 ± 1.266) and juice extract (16.937 ± 4.030) at a concentration of 62,50 µg/ml were next. At a concentration of 250 µg/ml, the root extracts demonstrated important DPPH inhibitory activity in ethanol (75.420 ± 0.469) and acetone (72.453 ± 0.425) extracts. Leaf water extract (46.123 ± 2.738) at 1000 µg/ml was demonstrated in **table 3 and figure 8**, respectively, as compared to ascorbic acid (86.817 ± 1.266) at 1000 µg/ml. These comparisons show that acetone (45.277 ± 3.068), ethanol (29.020 ± 0.701) and water (14.623 ± 1.454) extracts all have substantial DPPH scavenging action even at low concentrations (15.63 µg/ml) in contrast with ascorbic acid (6.730 ± 0.849).

Table 2: Quantitative test for the scavenging of free radicals by 2,2-diphenyl-1-picrylhydrazyl (DPPH) leaves extracts

	DPPH inhibition (%) ± SEM				
Concentration (µg/ml)	Acetone extract	Ethanol extract	Water extract	Juice extract	Ascorbic acid
0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
15.63	1.393 ± 0.173	19.183 ± 1.979	3.290 ± 1.558	7.230 ± 2.187	6.730 ± 0.849
31.25	11.303 ± 1.073	29.003 ± 2.692	5.587 ± 1.194	16.143 ± 2.801	14.080 ± 3.283
62.50	15,963 ± 0.553	27.000 ± 8.783	5.430 ± 0.712	16.937 ± 4.030	38.313 ± 0.861
125.00	12.917 ± 1.931	44.537 ± 2.215	15.273 ± 1.221	7.868 ± 5.601	82.983 ± 1.578
250.00	4.983 ± 0.590	55.070 ± 1.816	11.093 ± 0.554	8.291 ± 2.744	85.143 ± 1.486
500.00	25.373 ± 0.875	63.740 ± 0.656	30.280 ± 3.070	0.652 ± 0.021	85.867 ± 1.166
1000.00	53.320 ± 3.381	65.337 ± 1.208	39.383 ± 1.035	1.081 ± 0.208	86.817 ± 1.266

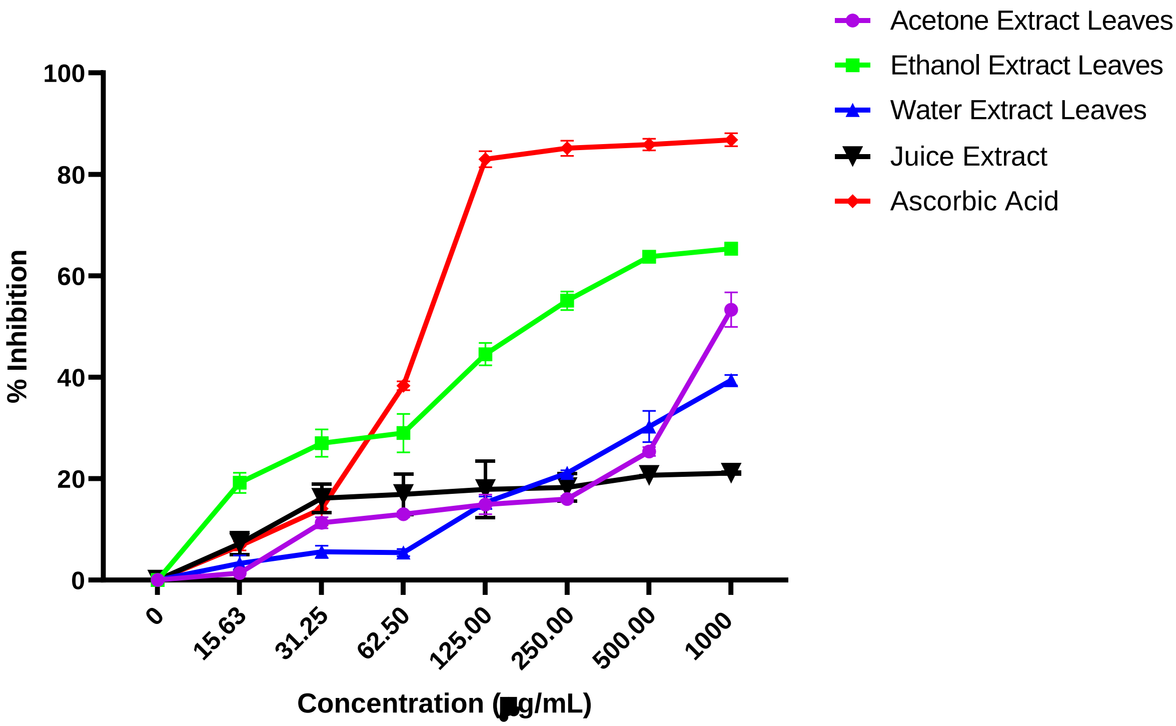

Figure 7: DPPH scavenging activity of *C. edulis* leaves acetone (AE), ethanol (EE), water (WE) and juice (JE) extracts. The statistical difference was found to be (* p < 0.1) between acetone and water extracts, (*p < 0.01) juice extract and ethanol showed no statistical difference as compared to ascorbic acid (positive control).

Table 3: Quantitative test for the scavenging of free radicals by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) roots extracts.

	DPPH inhibition (%) ± SEM			
Concentration (µg/ml)	Acetone extract	Ethanol extract	Water extract	Ascorbic acid
0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
15.63	45.277 ± 3.068	29.020 ± 0.701	14.623 ± 1.454	6.730 ± 0.849
31.25	49.183 ± 0.941	36.087 ± 6.939	28.027 ± 0.627	14.080 ± 3.283
62.50	55.517 ± 0.720	53.840 ± 6.098	10.583 ± 0.826	38.313 ± 0.861
125.00	53.879 ± 1.193	48.373 ± 1.006	19.717 ± 9.091	82.983 ± 1.578
250.00	72.453 ± 0.425	75.420 ± 0.469	24.883 ± 1.826	85.143 ± 1.486
500.00	71.930 ± 1.052	74.437 ± 0.127	24.417 ± 5.554	85.867 ± 1.166
1000.00	65.733 ± 1.679	64.33 ± 3.276	46.123 ± 2.738	86.817 ± 1.266

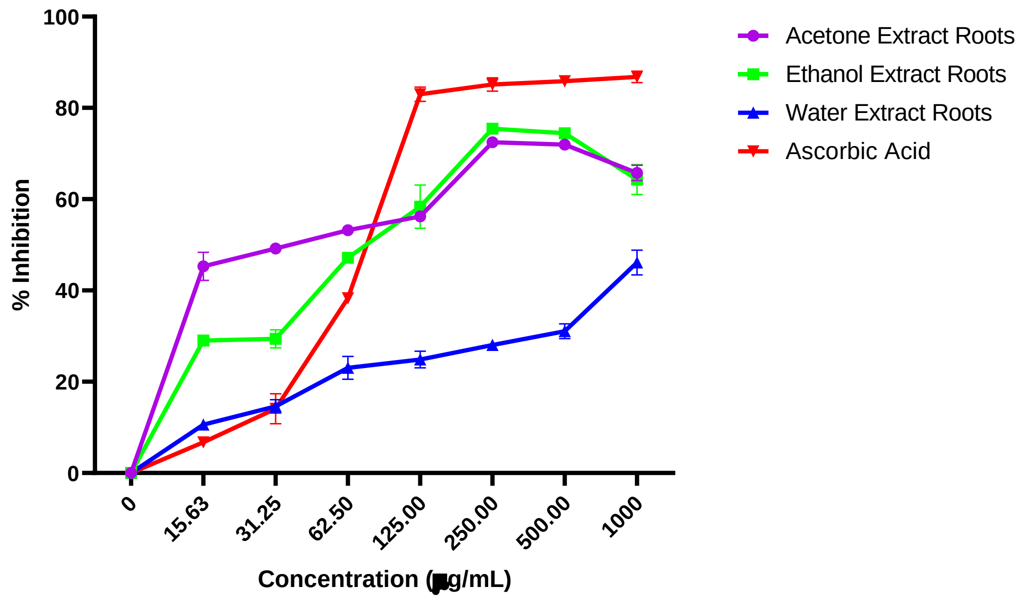

Figure 8: DPPH scavenging activity of *C. edulis* roots acetone (AE), ethanol (EE) and water (WE) extracts. The acetone and ethanol extracts showed no significant difference while water extract showed statistical significance of (**$p < 0.01$) as compared to ascorbic acid (positive control).

4.4. Quantitative test by ferric reducing antioxidant power (FRAP) assay

Polyphenols are significant plant chemicals that are well recognised to have a wide range of biological activities, including antioxidant properties (Omoruyi *et al.,* 2012). Its redox characteristics, hydrogen donors, and singlet oxygen reducing agents (Gülçin *et al.,* 2011), which can be useful in adsorbing and neutralizing free radicals, are primarily responsible for their antioxidant action (Gülçin *et al.,* 2011). Scientists, food producers, and consumers are becoming more and more interested in the role that antioxidant components of plant materials play in maintaining health and preventing cancer and heart disease (Yazdanparast *et al.,* 2008). By evaluating the conversion of Fe^{3+}/ferricyanide complexes to Fe^{2+}/ferrous form, the antioxidant properties of the *C. edulis* extract from both leaves and roots were examined (Mohamed *et al.,* 2008). The greatest reducing power in the leaves of *C. edulis* extracts, as shown in **table 4 and figure 9**, was demonstrated at a concentration of 1000 µg/ml in ethanol (4118.756 ± 204.925), acetone

(3261.324 ± 363.379), juice (1897.928 ± 40.995), and water (1165.870 ± 55.795) extracts, as opposed to ascorbic acid (4153.073 ± 67.753). According to **table 5 and figure 10**, the highest reducing power of *C. edulis* extracts in roots was discovered to be in acetone (4422.065 ± 343.429), ethanol (2799.964 ± 99.501), water (1793.361 ± 19.152) as compared to ascorbic acid (4153.073 ± 67.753). There may be hydrophilic polyphenolic chemicals present, which would explain the plant extracts reported lowering ability (Omoruyi *et al.,* 2012). These findings are completely consistent with earlier research that suggested a relationship between the reducing capacity of plant extracts and their phenolic content (Park *et al.,* 2010).

Table 4: Antioxidant efficacy reduced by ferric ions (FRAP) leaves extracts.

	Ferric reducing antioxidant power (%) ± SEM				
Concentration (µg/ml)	Acetone extract	Ethanol extract	Water extract	Juice extract	Ascorbic acid
0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
15.63	10.896 ± 2.657	112.051 ± 28.608	5.329 ± 4.065	4.919 ± 0.974	6.333 ± 1.553
31.25	64.060 ± 15.367	342.042 ± 37.732	19.774 ± 6.550	8.718 ± 1.554	22.389 ± 2.859
62.50	201.295 ± 18.979	772.314 ± 48.179	49.510 ± 25.249	35.889 ± 1.027	87.969 ± 1.872
125.00	525.276 ± 49.322	1155.693 ± 124.672	153.948 ± 42.175	174.270 ± 7.475	457.602 ± 6.669
250.00	777.549 ± 58.314	2068.978 ± 144.446	290.911 ± 27.408	428.342 ± 11.079	1038.541 ± 8.507
500.00	1324.114 ± 42.848	4029.371 ± 264.914	746.692 ± 56.182	888.354 ± 21.070	2251.690 ± 38.437
1000.00	3261.324 ± 363.379	4118.756 ± 204.925	1165.870 ± 55.795	1897.928 ± 40.995	4153.073 ± 67.753

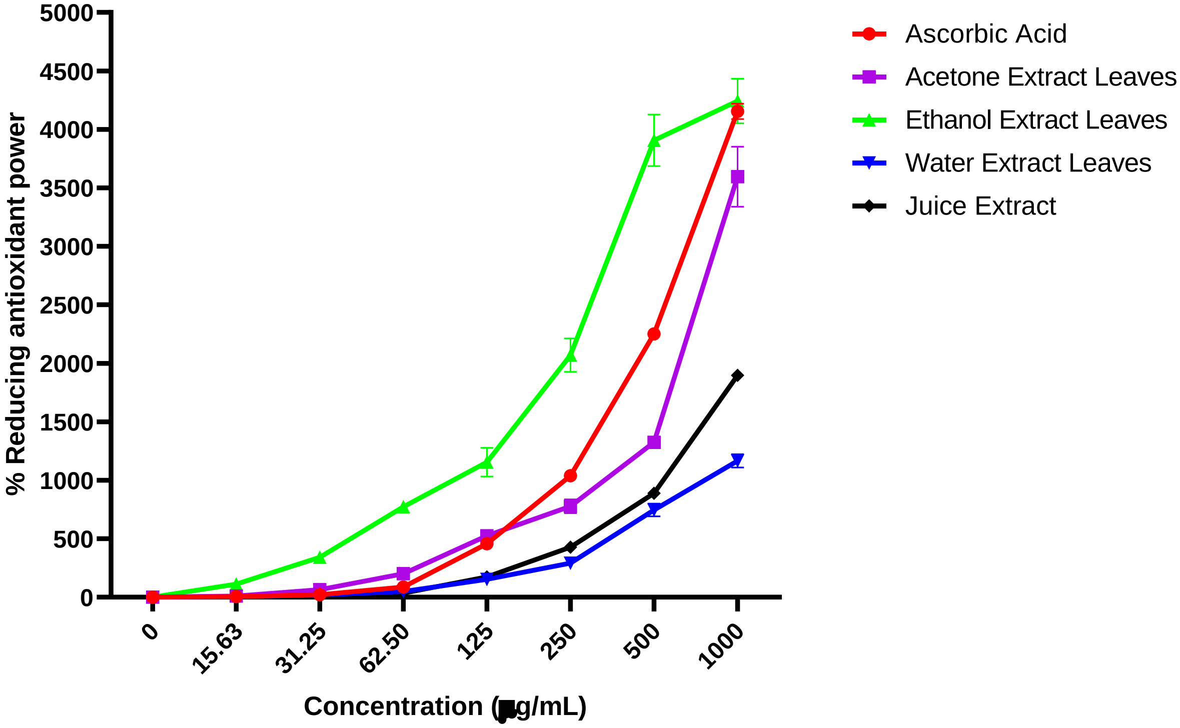

Figure 9: Reducing antioxidant activity of *C. edulis* leaves acetone (AE), ethanol (EE), water (WE) and juice (JE) extracts. Acetone and ethanol extracts showed no significant while water and juice extracts showed statistical significance of (*$p < 0.01$) as compared to ascorbic acid (positive control).

Table 5: Antioxidant efficacy reduced by ferric ions (FRAP) roots extracts.

	Ferric reducing antioxidant power (%) ± SEM			
Concentration (µg/ml)	Acetone extract	Ethanol extract	Water extract	Ascorbic acid
0	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000	0.000 ± 0.000
15.63	157.778 ± 5.757	246.501 ± 25.904	69.575 ± 39.048	6.333 ± 1.553
31.25	305.234 ± 5.649	484.448 ± 21.824	135.161 ± 46.537	22.389 ± 2.859
62.50	577.762 ± 21.245	985.874 ± 37.773	193.184 ± 52.993	87.969 ± 1.872
125.00	1086.572 ± 59.819	2110.068 ± 60.618	302.490 ± 50.688	457.602 ± 6.669
250.00	2556.326 ± 167.072	2949.907 ± 59.041	515.838 ± 43.024	1038 ± 8.507
500.00	4109.345 ± 62.711	3101.318 ± 53.789	956.091 ± 53.349	2251.690 ± 38.437
1000.00	4422.065 ± 343.429	2799.964 ± 99.501	1793.361 ± 19.152	4153.073 ± 67.753

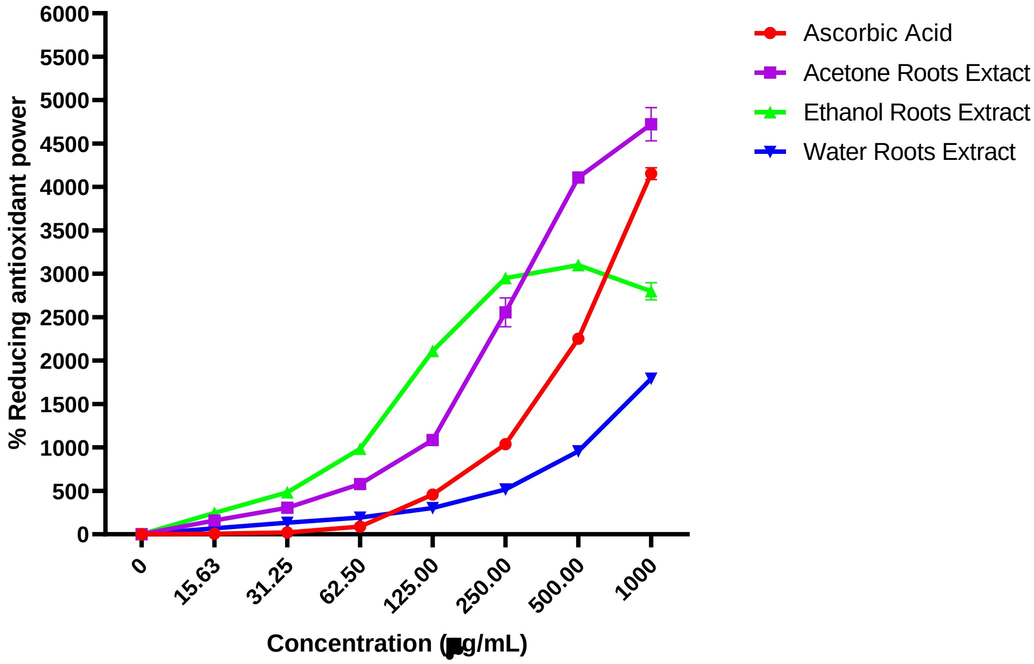

Figure 10: Reducing antioxidant activity of *C. edulis* roots acetone (AE), ethanol (EE) and water (WE) extracts. Acetone and water extracts showed no statistical significance while water extract showed statistical significance of (**$p < 0.01$) as compared to ascorbic acid (positive control).

4.5. Compounds composition of *C. edulis* AE, EE and WE by Ultra-high performance liquid chromatography-Mass spectrophotometry (UHPLC-MS) Analysis.

The phytochemical compounds in *C. edulis* root extracts were investigated using a high-resolution UHPLC–MS-based method. **Table 6** shows results obtained from the UHPLC and appendix D shows the chromatographs obtained from UHPLC. Three compounds were found in acetone root extract (15-methylhexadecasphing-4-enine; methyl stearate and ethyl 2-[4,7-bis(2-ethoxy-2-oxoethyl)-9-(3-hydroxypropyl)-8-[methylsulfonyl(octyl)amino]-1,4,7,10-tetrazacyclododec-1-yl]acetate), thirteen compounds were present in ethanol root extract ((1R)-1-[4-[(3S,5R)-4-[2-[(1R)-1-hydroxyethyl]pyrimidin-4-yl]-3,5-dimethylpiperazin-1-yl]pyrimidin-2-yl]ethanol; 2-N-(4-aminocyclohexyl)-9-cyclopentyl-6-N-[4-[3-(dipropylamino)propyl]piperidin-1-yl]purine-

2,6-diamine; (1R)-N',N'-dimethyl-1-phenyl-N-[1-[3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]propyl]piperidin-4-yl]ethane-1,2-diamine; 2-hexadecylsulfonylethanol; 6-methyl-5-(2-piperazin-1-ylethylamino)-2H-1,2,4-triazin-3-one; Artemin; Gingerdiol; 2-[3-[4-(3-Aminopropylamino)butylamino]propylamino]ethanesulfonic acid; Bencyclane; 4-(Piperazin-1-yl)-2,6-di(pyrrolidin-1-yl)pyrimidine; 2,7-bis(3,5-dimethylphenyl)-9H-fluorene; Ganaxolone; methyl stearate and 3-Sulfanylideneoxolane-2,5-dione) while eleven compounds were present in water root extract ((1R)-N',N'-dimethyl-1-phenyl-N-[1-[3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]propyl]piperidin-4-yl]ethane-1,2-diamine; 1,1,1-Trinitropropan-2-yl prop-2-enoate; Artemin; Dehydroeffusol; N-(4,5-dihydro-1H-imidazol-2-ylmethyl)-2-(1H-1,2,4-triazol-5-yl)aniline; 2-isobutyl-4-(5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid; 2-[4-[[(5-tert-butyl-1,3,4-thiadiazol-2-yl)-methylamino]methyl]piperidin-1-yl]-N-methylacetamide; Linoleic Acid; 1,12-Dodecanediamine, N,N'-bis(4,5-dihydro-1H-imidazol-2-yl)-141951-28-2; 3-Hexyl-2-[5-(2-methylbutan-2-yl)-4-(5-methylideneoctyl)thiophen-2-yl]-5-(2-methyloctan-2-yl)thiophene and Thiolane-2,3,4-trione). Methyl stearate was found in both acetone and ethanol extracts; however, their biological activities remain unknown. Artemin was also found in both ethanol and water extracts, and its biological activity remains unknown as well. (1R)-N',N'-dimethyl-1-phenyl-N-[1-[3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]propyl]piperidin-4-yl]ethane-1,2-diamine was present in both ethanol and water extracts respectively, which is known as neurotransmitter. Thiolane-2,3,4-trione which is an isomer of 3-Sulfanylideneoxolane-2,5-dione was also detected in both ethanol and water extract, which its biological activity remains unknown. Linolenic acid was only found in water extract of *C. edulis* which is the only compound reported in other Aizoaceae family (*A. canariense*) [Freije et al., 2013]. This compound (linolenic acid) was used for inflammatory skin conditions such as psoriasis and eczema. Twenty-six of the twenty-seven compounds were identified for the first time in *C. edulis* acetone, ethanol, and water extracts; the only exception being linolenic acid, which was described in the Aizoaceae family.

Table 6: Detection of phenolic compounds from *C. edulis* acetone, ethanol and water extract by UHPLC–MS

Acetone extract						
Compound	Retention time (min)	Mass	Formula	Biological activities	Compound CID	Reference
15-methylhexadecasphing-4-enine	8.695	285.5	C17H35NO2	Unknown	70798	
Methyl Stearate	25.010	298.5	C19H38O2	Unknown	8907	
ethyl 2-[4,7-bis(2-ethoxy-2-oxoethyl)-9-(3-hydroxypropyl)-8-[methylsulfonyl(octyl)amino]-1,4,7,10-tetrazacyclododec-1-yl]acetate	26.894	693.9	C32H63N5O9S	Unknown	67599754	

Ethanol extract

Compound	Retention time (min)	Mass	Formula	Biological activities	Compound CID	Reference
(1R)-1-[4-[(3S,5R)-4-[2-[(1R)-1-hydroxyethyl]pyrimidin-4-yl]-3,5-dimethylpiperazin-1-yl]pyrimidin-2-yl]ethanol	8.857	358.4	C18H26N6O2	Anti-diabetic	9841731	(Ng et.al., 1998)
2-N-(4-aminocyclohexyl)-9-cyclopentyl-6-N-[4-[3-(dipropylamino)propyl]piperidin-1-yl]purine-2,6-diamine	10.567	539.8	C30H53N9	Unknown	70277049	
(1R)-N',N'-dimethyl-1-phenyl-N-[1-[3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]propyl]piperidin-4-yl]ethane-1,2-diamine	14.125	471.6	C28H37N7	Neurotransmitter	10504600	(Yohn et.al., 2017)
2-hexadecylsulfonylethanol	14.549	334.6	C18H38O3S	Unknown	292509	
6-methyl-5-(2-piperazin-1-ylethylamino)-2H-1,2,4-triazin-3-one	15.959	238.29	C10H18N6O	Unknown	3283592	
Artemin	17.350	266.33	C15H22O4	Unknown	3678270	

Gingerdiol	19.257	296.4	C17H28O4	Anthelmintic activity	11369949	(Márquez-Navarro et.al., 2009)
2-[3-[4-(3-Aminopropylamino)butylamino]propylamino]ethanesulfonic acid	20.085	310.46	C12H30N4O3S	Unknown	91062258	
Bencyclane	21.771	289.5	C19H31NO	A receptor antagonist	2312	(Yasgar et.al., 2023)
4-(Piperazin-1-yl)-2,6-di(pyrrolidin-1-yl)pyrimidine	22.575	302.42	C16H26N6	autoimmune and inflammation	14585243	(National Centre for Biotechnology Information, 2023)
2,7-bis(3,5-dimethylphenyl)-9H-fluorene	23.470	374.5	C29H26	Unknown	59561022	

Ganaxolone	24.646	332.5	C22H36O2	Anxiolytic, analgesic, anticonvulsant, sedative, hypnotic, and anaesthetic properties.	6918305	(Lingamaneni et.al., 2003)
Methyl Stearate	25.023	298.5	C19H38O2	Unknown		
3-Sulfanylideneoxolane-2,5-dione	28.417	130.12	C4H2O3S	Unknown		

Water extract

Compound	Retention time (min)	Mass	Formula	Biological activities	Compound CID	Reference

(1R)-N',N'-dimethyl-1-phenyl-N-[1-[3-[5-(1,2,4-triazol-4-yl)-1H-indol-3-yl]propyl]piperidin-4-yl]ethane-1,2-diamine	14.116	471.6	C28H37N7	neurotransmitter	10504600	(Yohn et.al., 2017)
1,1,1-Trinitropropan-2-yl prop-2-enoate	15.948	249.14	C6H7N3O8	Unknown	87856317	
Artemin	17.354	266.33	C15H22O4	Unknown	3678270	
Dehydroeffusol	18.046	250.29	C17H14O2	Anticancer	5316810	(Ribera-Fonseca et.al., 2020)
N-(4,5-dihydro-1H-imidazol-2-ylmethyl)-2-(1H-1,2,4-triazol-5-yl)aniline	19.251	242.28	C12H14N6	Adrenergic receptor	22493236	(Ruan et.al., 1998)
2-isobutyl-4-(5-phenyl-1H-pyrrolo[2,3-b]pyridin-3-yl)benzoic acid	20.088	370.4	C24H22N2O2	Antihypertensive, anti-inflammatory, cardioprotective	45270042	(Gan et.al., 2018)
2-[4-[[(5-tert-butyl-1,3,4-thiadiazol-2-yl)-	22.006	339.5	C16H29N5OS	Unknown	71968117	

methylamino]methyl]piperidin-1-yl]-N-methylacetamide						
Linoleic Acid	23.445	280.4	C18H32O2	Agonist receptor Skin inflammation	5280450	(Rives et.al., 2018) (Freije et.al., 2013)
1,12-Dodecanediamine, N,N'-bis(4,5-dihydro-1H-imidazol-2-yl)- **141951-28-2**	24.390	336.5	C18H36N6	Antitrypanosomal activity	12004386	(Hoet et.al., 2004)
3-Hexyl-2-[5-(2-methylbutan-2-yl)-4-(5-methylideneoctyl)thiophen-2-yl]-5-(2-methyloctan-2-yl)thiophene	27.923	571.0	C37H62S2	Unknown	89101277	
Thiolane-2,3,4-trione	28.050	130.12	C4H2O3S	Unknown	22066345	

4.6. *In vitro* reduction of the viability of HeLa and CaSki cells by *C. edulis* extracts

The MTT assay (section 3.2.8) and the Muse^{TM} cell count & viability kit assay (section 3.2.8) were used to evaluate the impact *of C. edulis* root extracts on the viability and proliferation of HeLa, CaSki, and Hek-293 cells. Following treatment of the HeLa, CaSki, and Hek-293 (non-cancerous) cells with *C. edulis* root extracts (acetone, ethanol, and water) and curcumin (positive control for apoptosis), cell cytotoxicity was employed to assess the cells' capacity to survive. The acetone extract of *C. edulis* considerably enhanced the proliferation and viability of HeLa cells in a dose-dependent manner, as seen by **figure 11 and table 7**. The proliferation and viability of HeLa cells were greatly enhanced by the *C. edulis* ethanol extract in a dose-dependent manner, as seen by **figure 12 and table 8**. HeLa cells viability and proliferation were markedly suppressed by the *C. edulis* water extract in a dose-dependent manner, as seen by **figure 13 and table 9**. The minimum inhibitory concentration (IC_{50}) was established at 1000 µg/ml. Curcumin reduced viability and proliferation of HeLa cells after 24 hours as shown in **figure 14** and **table 10** where IC_{50} was found to be at 40 µM.

As shown by **figure 15 and table 11**, the acetone extract of *C. edulis* significantly increased the viability and proliferation of CaSki cells in a dose-dependent manner. The *C. edulis* ethanol extract significantly increased the viability and proliferation of CaSki cells in a dose-dependent manner, as shown in figure **16 and table 12**. **Figure 17 and table 13** demonstrate how the *C. edulis* water extract significantly reduced CaSki cells viability and proliferation in a dose-dependent manner. A concentration of 1000 µg/ml was determined to be the lowest inhibitory concentration (IC_{50}). After a 24-hour period, curcumin decreased the viability and proliferation of CaSki cells, as seen in **figure 18 and table 14**, where an IC_{50} of 50 µM was determined. An IC_{50} of 1000 µg/ml that was found on both cell lines (HeLa and CaSki) did not have any significant impact on non-cancerous cells (Hek-293), which is shown in **figure 19 and table 15** respectively.

Table 7: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of HeLa cells with various concentrations of *C. edulis* acetone extract after 72 hours

C. edulis acetone extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	95,148 ± 2.214
15.63 µg/ml	116.883 ± 9.836
31.25 µg/ml	122.418 ± 9.809 ***
62.50 µg/ml	149.007 ± 1.974 ****
125.00 µg/ml	186.265 ± 3.862 ****
250.00 µg/ml	238.804 ± 10.518 ****
500.00 µg/ml	334.713 ± 1.443 ****
1000.00 µg/ml	346.046 ± 10.878 ****

**** indicates $p \leq 0.0001$ and *** indicates $p \leq 0.001$

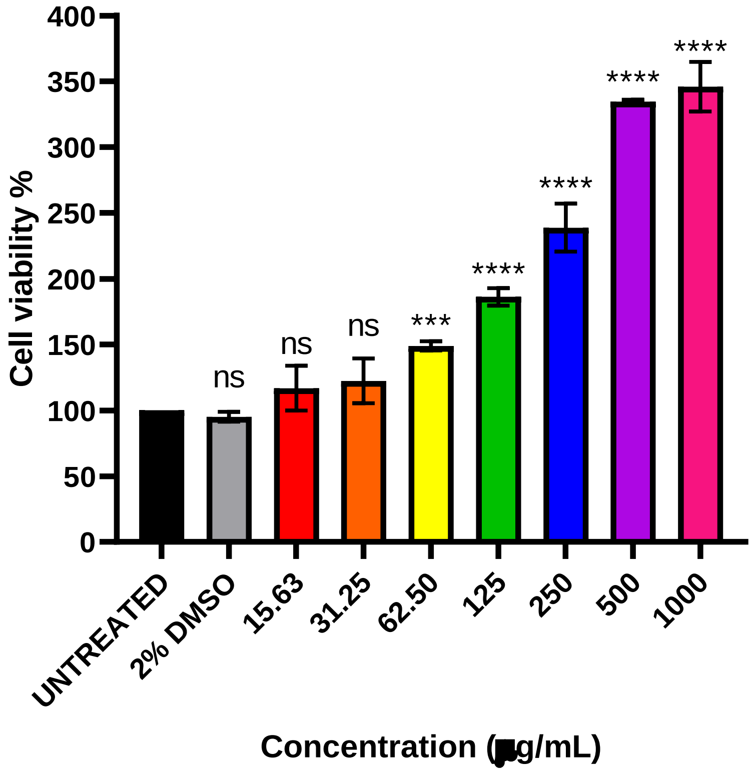

Figure 11: Effect of *C. edulis* acetone extract on the viability of HeLa cells.

Acetone extract significantly ($p \leq 0.001$ *** and $p \leq 0.0001$ ****) increased the viability of the HeLa cells in a concentration-dependent manner.

Table 8: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of HeLa cells with various concentrations of *C. edulis* ethanol extract after 72 hours

C. edulis ethanol extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	91.468 ± 0.398
15.63 µg/ml	220.518 ± 2.831 ****
31.25 µg/ml	223.414 ± 2.619 ****
62.50 µg/ml	228.726 ± 4.612 ****
125.00 µg/ml	232.141 ± 6.495 ****
250.00 µg/ml	241.965 ± 6.862 ****
500.00 µg/ml	250.247 ± 5.884 ****
1000.00 µg/ml	256.848 ± 7.494 ****

**** indicates $p \leq 0.0001$

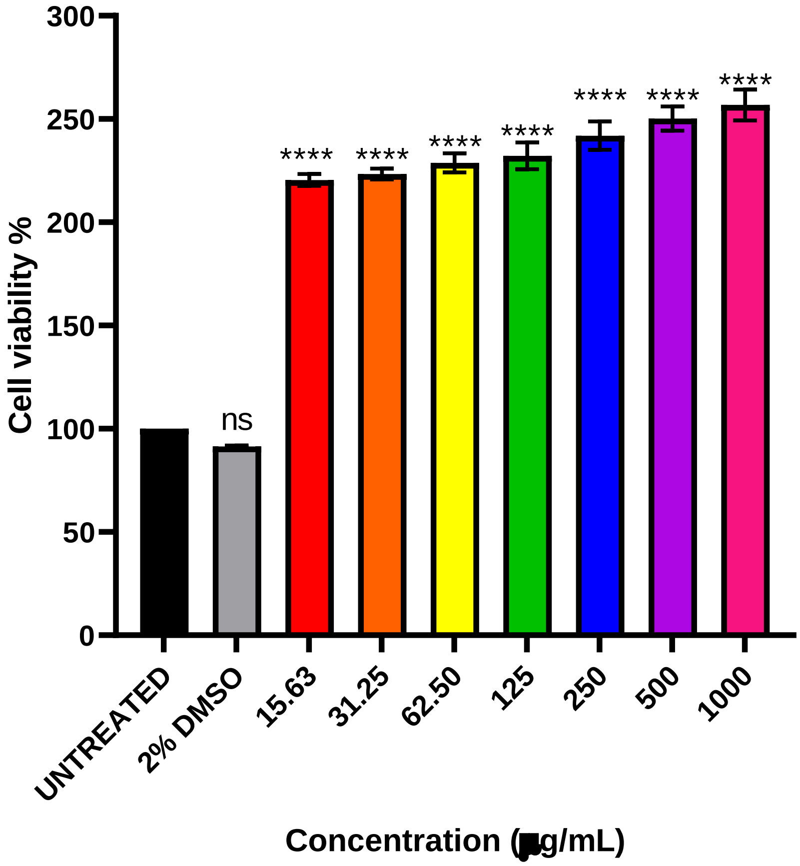

Figure 12: Effect of *C. edulis* ethanol extract on the viability of HeLa cells.

Ethanol extract significantly ($p \leq 0.0001$ ****) increased the viability of the HeLa cells in a concentration-dependent manner.

Table 9: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of HeLa cells with various concentrations of *C. edulis* water extract after 72 hours

C. edulis water extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% H_2O)	93.529 ± 1.745
15.63 µg/ml	112.108 ± 5.939
31.25 µg/ml	111.892 ± 6.104
62.50 µg/ml	106.227 ± 5.876
125.00 µg/ml	101.956 ± 5.321
250.00 µg/ml	93.574 ± 1.391
500.00 µg/ml	82.871 ± 1.565 *
1000.00 µg/ml	53.821 ± 3.115 ****

**** indicates $p \leq 0.0001$ and * indicates $p \leq 0.1$

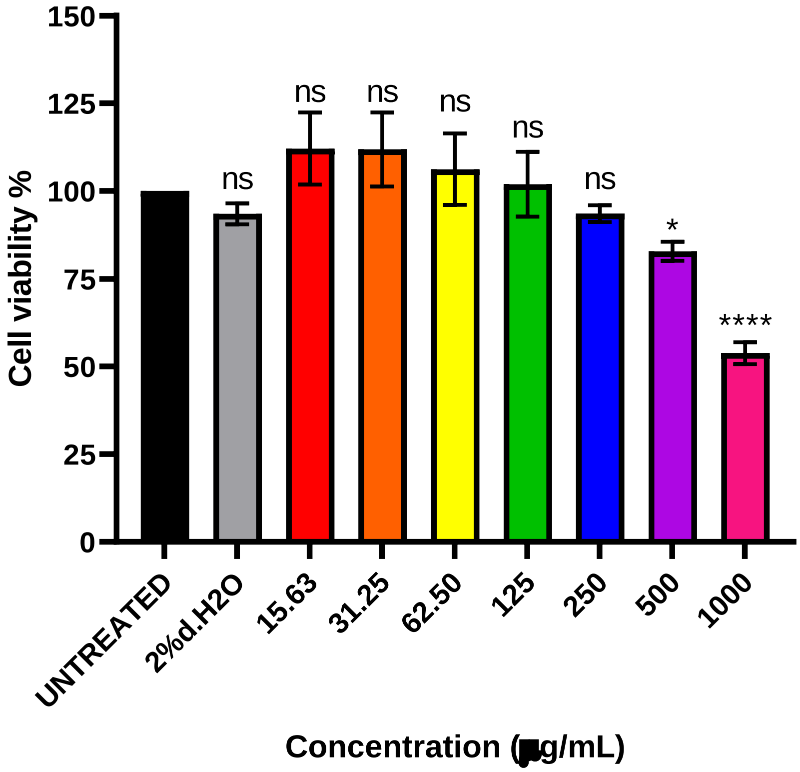

Figure 13: Effect of *C. edulis* water extract on the viability of HeLa cells.

Water extract significantly ($p \leq 0.0001$ **** and * $p \leq 0.1$) reduced the viability of the HeLa cells in a concentration-dependent manner.

Table 10: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of HeLa cells with various concentrations of curcumin after 24 hours

Curcumin	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	95.333 ± 3.055
10 µM	90.000 ± 3.464 **
20 µM	80.333 ± 2.517 ****
30 µM	69.667 ± 3.786 ****
40 µM	51.667 ± 1.528 ****
50 µM	42.333 ± 0.577 ****
60 µM	37.667 ± 1.555 ****

**** indicates p ≤ 0.0001 and ** indicates p ≤ 0.01

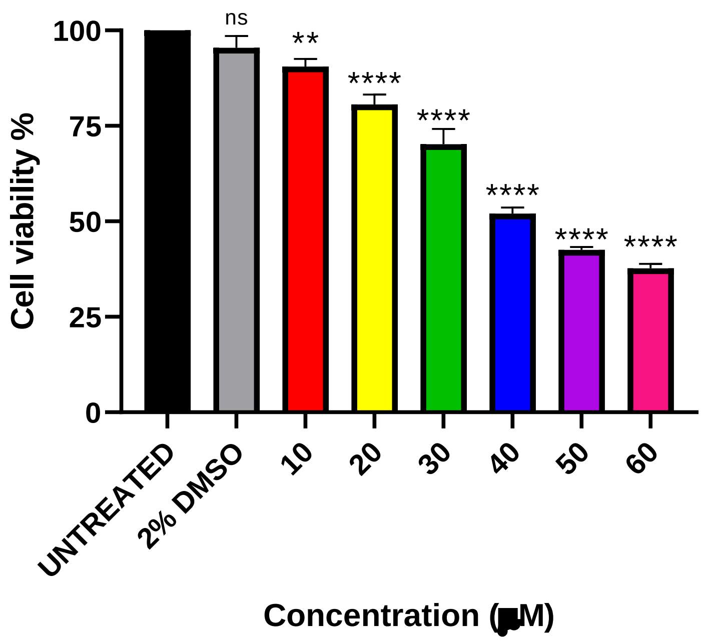

Figure 14: Effect of curcumin on the viability of HeLa cells.

Curcumin significantly ($p \leq 0.0001$ **** and ** $p \leq 0.01$) reduced the viability of the HeLa cells in a concentration-dependent manner.

Table 11: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of CaSki cells with various concentrations of *C. edulis* acetone extract after 72 hours

C. edulis acetone extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	93.516 ± 0.968
15.63 µg/ml	100.216 ± 3.924
31.25 µg/ml	101.831 ± 2.662
62.50 µg/ml	108.841 ± 0.324
125.00 µg/ml	109.489 ± 0.610
250.00 µg/ml	115.249 ± 2.815 **
500.00 µg/ml	115.882 ± 3.059 **
1000.00 µg/ml	138.006 ± 5.105 ****

**** indicates $p \leq 0.0001$ and ** indicates $p \leq 0.01$

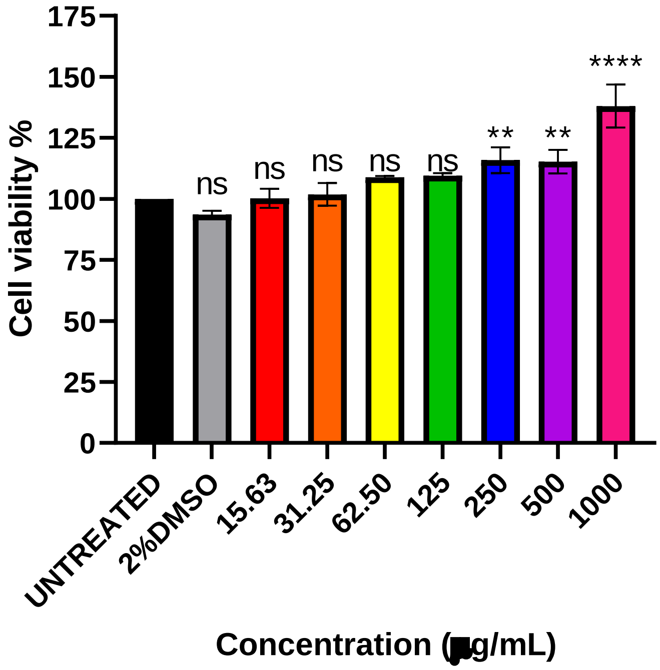

Figure 15: Effect of *C. edulis* acetone extract on the viability of CaSki cells.

C. edulis acetone extract significantly ($p \leq 0.0001$ **** and ** $p \leq 0.01$) increased the viability of the CaSki cells in a concentration-dependent manner.

Table 12: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of CaSki cells with various concentrations of *C. edulis* ethanol extract after 72 hours

C. edulis ethanol extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	92.993 ± 1.497
15.63 µg/ml	142.290 ± 6.101 *
31.25 µg/ml	143.478 ± 5.434 **
62.50 µg/ml	146.410 ± 8.476 **
125.00 µg/ml	149.530 ± 9.717 **
250.00 µg/ml	148.483 ± 10.011 **
500.00 µg/ml	149.229 ± 11.521 **
1000.00 µg/ml	155.508 ± 11.878 **

** indicates $p \leq 0.01$ and * indicates $p \leq 0.1$

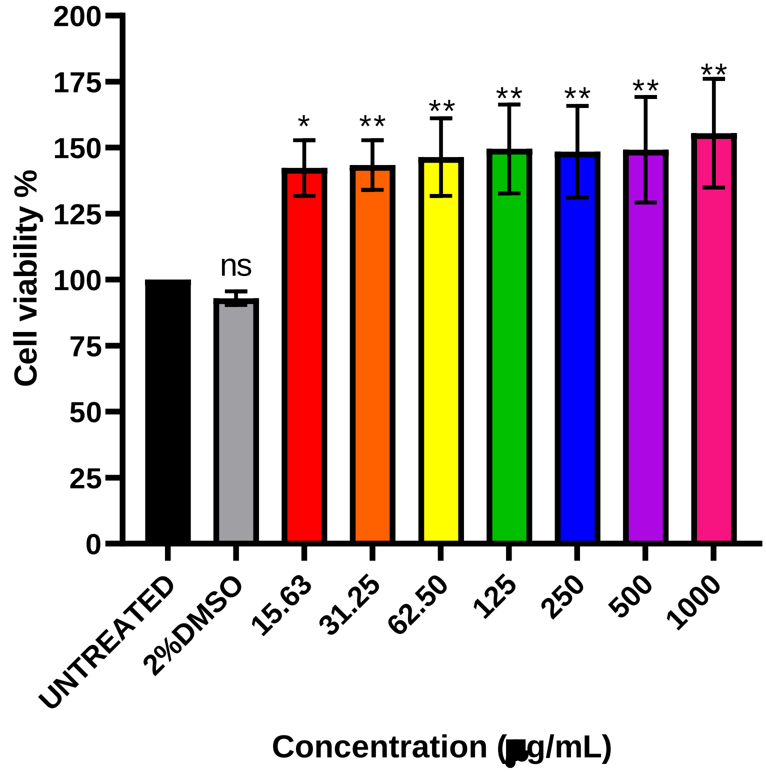

Figure 16: Effect of *C. edulis* ethanol extract on the viability of CaSki cells.

C. edulis ethanol extract significantly ($p \leq 0.01$ ** and * $p \leq 0.1$) increased the viability of the CaSki cells in a concentration-dependent manner.

Table 13: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of CaSki cells with various concentrations of *C. edulis* water extract after 72 hours

C. edulis water extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	93.529 ± 1.745
15.63 µg/ml	112.108 ± 5.939
31.25 µg/ml	106.227 ± 6.104
62.50 µg/ml	106.227 ± 5.876
125.00 µg/ml	101.956 ± 5.321
250.00 µg/ml	93.574 ± 1.391
500.00 µg/ml	82.871 ± 1.565 *
1000.00 µg/ml	53.821 ± 3.115 ****

**** indicates $p \leq 0.0001$ and * indicates $p \leq 0.1$

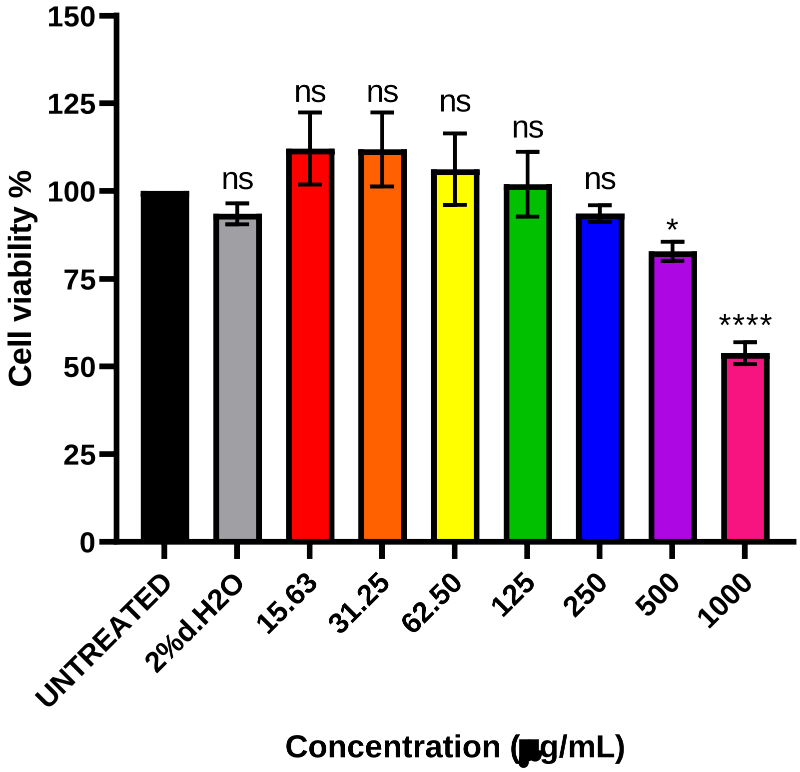

Figure 17: Effect of *C. edulis* water extract on the viability of CaSki cells.

C. edulis water extract significantly ($p \leq 0.0001$ **** and * $p \leq 0.1$) reduced the viability of the HeLa cells in a concentration-dependent manner.

Table 14: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of CaSki cells with various concentrations of curcumin after 24 hours

Curcumin	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% DMSO)	91.000 ± 6.928
10 µM	76.333 ± 2.887 ***
20 µM	68.333 ± 2.887 ****
30 µM	63.667 ± 2.309 ****
40 µM	57.667 ± 2.309 ****
50 µM	51.803 ± 1.969 ****
60 µM	43.770 ± 1.663 ****

**** indicates $p \leq 0.0001$ and *** indicates $p \leq 0.001$

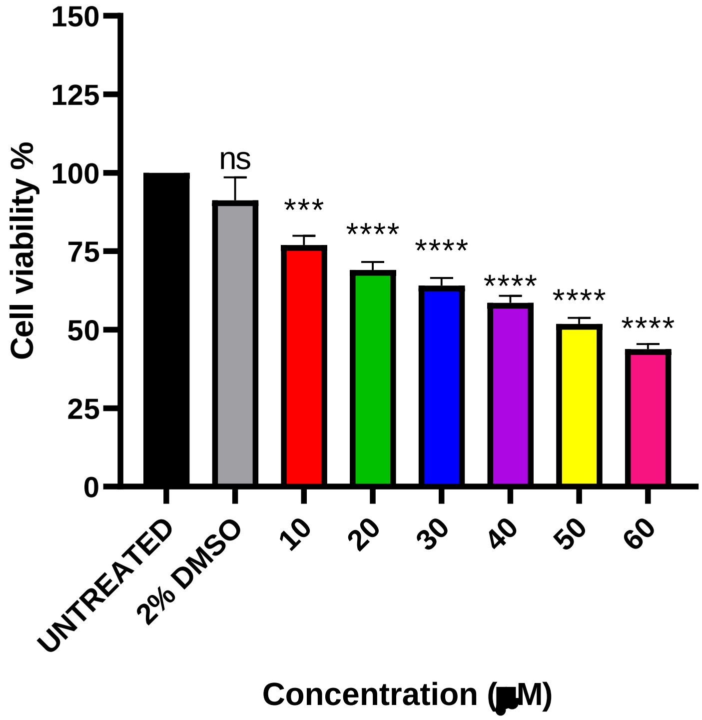

Figure 18: Effect of curcumin on the viability of CaSki cells.

Curcumin significantly (p ≤ 0.0001 **** and *** p≤ 0.001) reduced the viability of the CaSki cells in a concentration-dependent manner.

Table 15: Cell viability average percentages ± standard error of the mean (SEM) after the treatment of Hek-293 cells with an IC_{50} of 1000 µg/ml concentration of *C. edulis* root water extract after 72 hours

C. edulis water extract treatment	Mean (%) ± SEM
Untreated	100.00 ± 0.000
Solvent control (2% d.H_2O)	97.243 ± 0.559
1000 µg/ml	97.187 ± 0.749

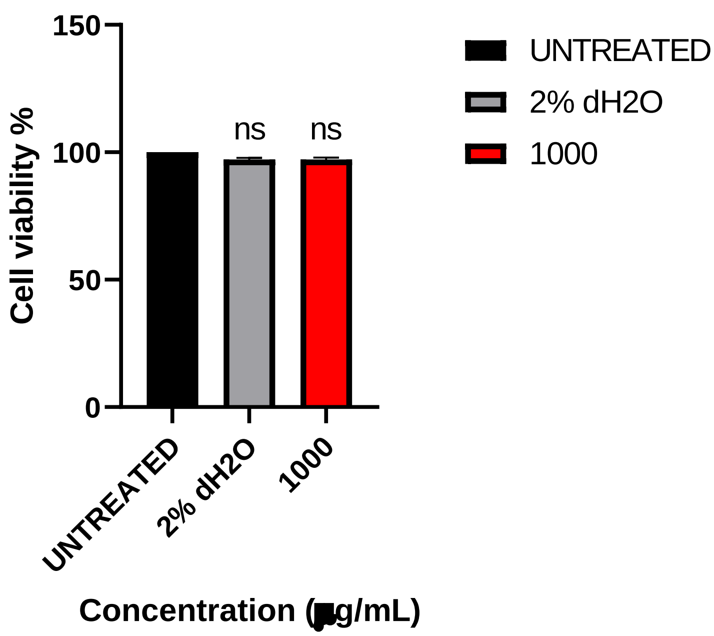

Figure 19: Effect of 1000 µg/ml (IC_{50}) on the viability of Hek-293 cells.

A concentration of 1000 µg/ml of *C. edulis* root water extract did not have a significant impact on non-cancerous cells (Hek-293).

4.6.1. Confirmation of *C. edulis* root water extract on cell viability in HeLa, CaSki and Hek-293 cells using Muse® Cell Analyzer

MTT assay results **(Figure 13, 17 and 19)** were confirmed by the Muse® Cell Analyzer **(Figure 21 A-C, 23 A-C and 25 A-C)**, which revealed that 1000 µg/ml of *C. edulis* root extract had an effect in reducing the viability of both HeLa (50.4 % with a $p \leq 0.0001$) and CaSki (49.3 % with a $p \leq 0.0001$) cells while the extract had no effect on non-cancerous Hek-293 cells. The same results from three independent experiments were represented graphically in **figure (20, 22 and 24)** and summarised in **table (16, 17 and 18)** respectively.

Table 16: Cell viability average percentages ± SEM after the treatment of HeLa cells with *C. edulis* root water extract of 1000 µg/ml

Treatments	Mean (%) ± SEM	
	Live	Dead
Untreated	95.367 ± 1.202	4.633 ± 1.202
Solvent control (2% d.H_2O)	92.733 ± 0.754	7.267 ± 0.754
1000 µg/ml (WE)	50.400 ± 0.493 ****	49.600 ± 1.365 ****

**** indicates $p \leq 0.0001$

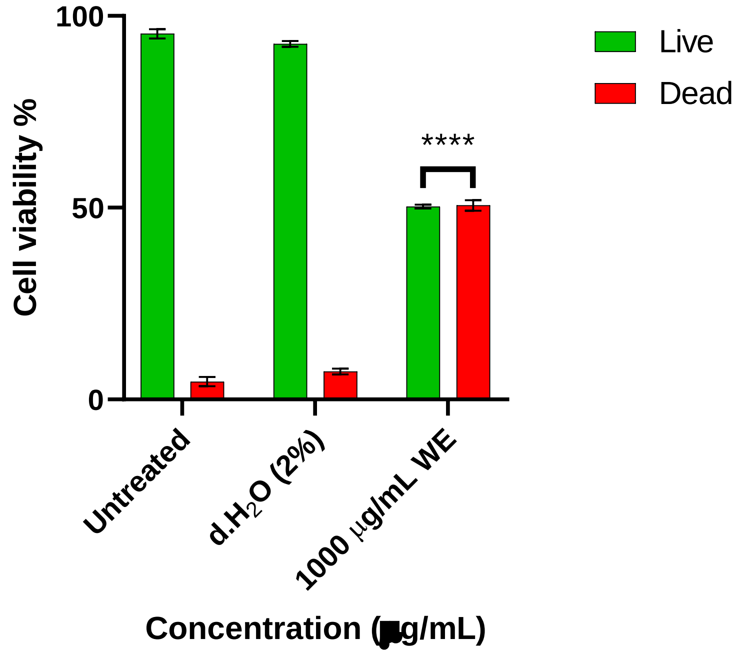

Figure 20: Confirmation of percentage viability of HeLa cells in response to treatment with *C. edulis* root water extract.

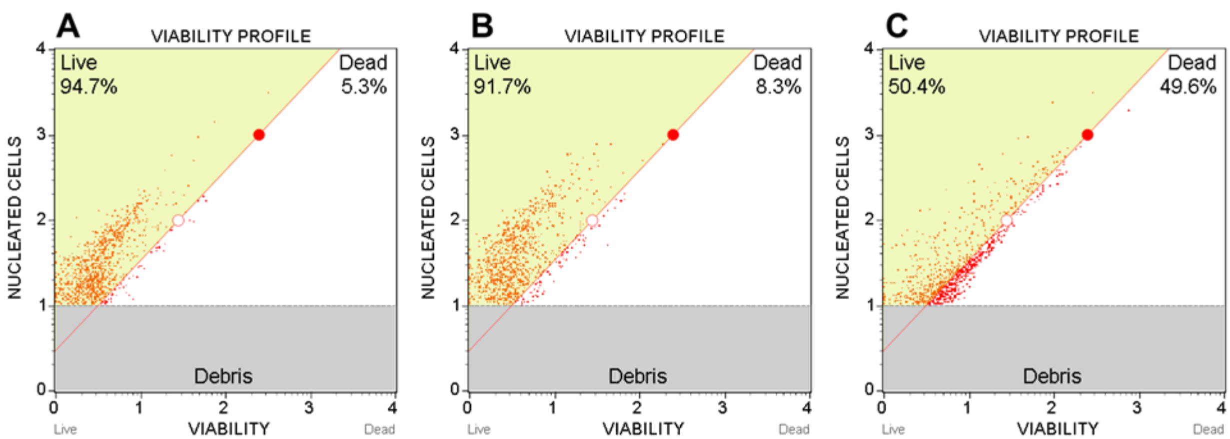

Figure 21: A-C: Analysis of % cell viability in HeLa cells using the Muse™ Count & Viability Kit.

The untreated (21 A), solvent control (21 B) were compared to *C. edulis* root water extract of 1000 µg/ml (21 C). The treatment reduced the viability of HeLa cells.

Table 17: Cell viability average percentages ± SEM after the treatment of CaSki cells with *C. edulis* root water extract of 1000 µg/ml

Treatments	Mean (%) ± SEM	
	Live	Dead
Untreated	99.600 ± 0.153	0.400 ± 0.153
Solvent control (2% d.H_2O)	99.600 ± 0.153	0.400 ± 0.153
1000 µg/ml (WE)	49.333 ± 3.417 ****	50.333 ± 3.169 ****

**** indicates $p \leq 0.0001$

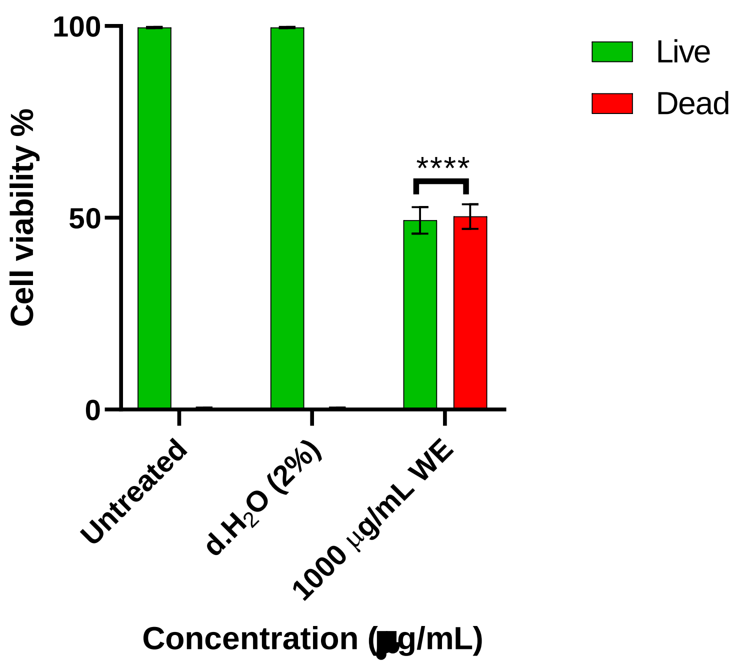

Figure 22: Confirmation of percentage viability of CaSki cells in response to treatment with *C. edulis* root water extract.

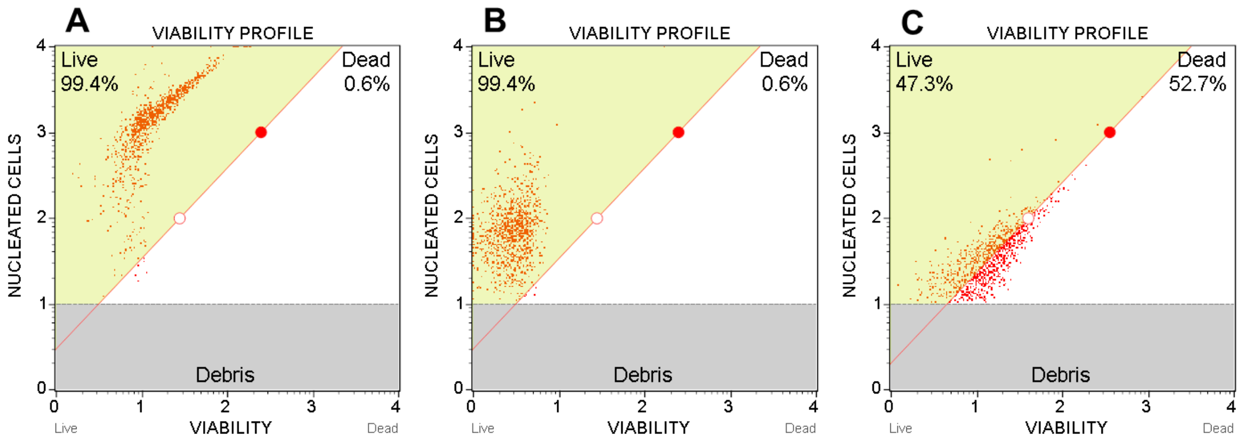

Figure 23: A-C: Analysis of % cell viability in CaSki cells using the Muse™ Count & Viability Kit.

The untreated (23 A), solvent control (23 B) were compared to *C. edulis* root water extract of 1000 µg/ml (23 C). The treatment reduced the viability of CaSki cells.

Table 18: Cell viability average percentages ± SEM after the treatment of Hek-293 cells with *C. edulis* root water extract of 1000 µg/ml

Treatments	Mean (%) ± SEM	
	Live	Dead
Untreated	99.467 ± 0.088	0.533 ± 0.088
Solvent control (2% d.H_2O)	99.433 ± 0.067	0.567 ± 0.067
1000 µg/ml (WE)	99.300 ± 0.252	0.700 ± 0.252

No-significant

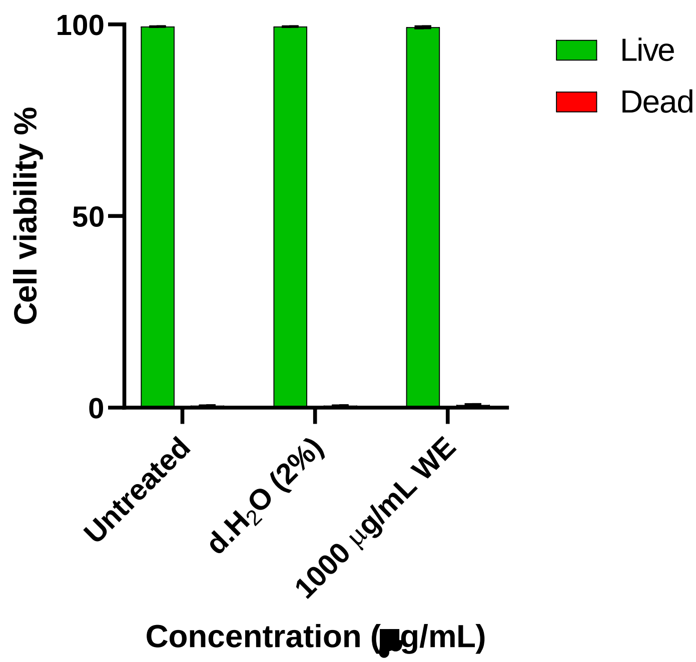

Figure 24: Confirmation of percentage viability of Hek-293 cells in response to treatment with *C. edulis* root water extract.

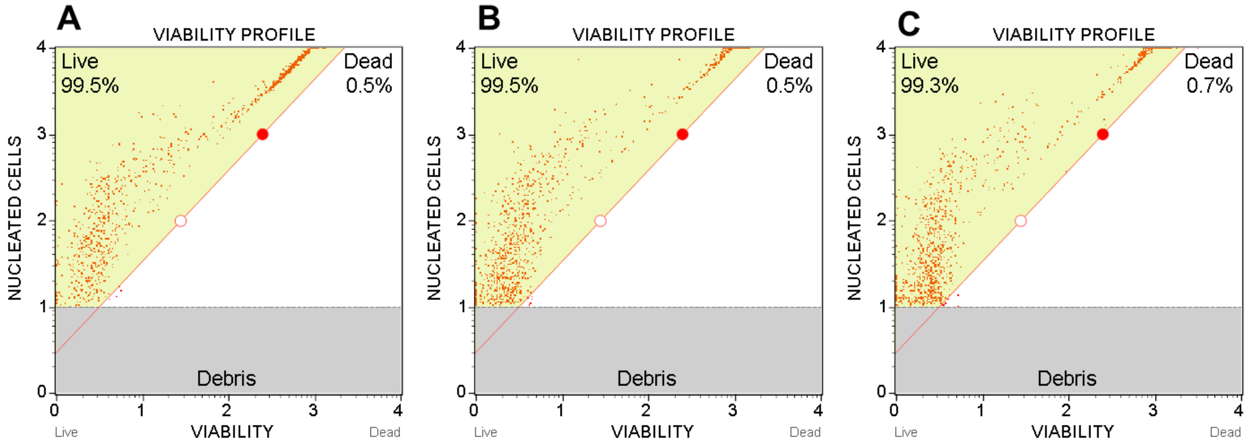

Figure 25: A-C: Analysis of % cell viability in Hek-293 cells using the Muse™ Count & Viability Kit.

The untreated (25 A), solvent control (25 B) were compared to *C. edulis* root water extract of 1000 µg/ml (25 C). The treatment had no significant change the viability of Hek-293 cells.

4.7. The effect of *C. edulis* root water extract on inducing apoptosis in HeLa and CaSki cells

The objective of this section was to investigate potential apoptotic activities of *C. edulis* against cervical cancer cells using the Annexin V analysis assay. The results of the cytotoxicity experiment (section 4.6) were supported by the use of the Muse™ Annexin V & Dead Cell analysis (section 3.2.9). In this assay, cells that are viable and do not exhibit apoptosis activity are labelled as "Live," cells that exhibit early apoptosis characteristics as "Early Apoptosis," cells that are dead and exhibit late apoptosis characteristics as "Late Apop/Dead," and cells that do not exhibit apoptosis activity are labelled as "Dead". The result from the Muse™ Annexin V & Dead Cell analysis showed that the treatment of with *C. edulis* root water extract (1000 µg/ml) on both (HeLa and CaSki cells) for 72 hours induced low apoptotic effects and curcumin (40 µM and 50 µM) for 24 hours significantly ($p \leq 0.0001$ ****) induced apoptosis as compared to untreated and solvent control cells respectively. The same trend was detected in the positive control (40 µM of curcumin) cells that exhibited around 24% positive cells for programmed cell death in (Figure 27 and

29). The similar results from three independent experiments were represented graphically (Figure 26) using the data summarized in table 19.

Table 19: Apoptosis average percentages ± SEM after the treatment of HeLa cells with *C. edulis* root water extract and curcumin.

Treatments	Mean (%) ± SEM			
	Live	Early apoptosis	Late apoptosis	Total apoptosis
Untreated	93.617 ± 0.713	3.817 ± 0.480	2.567 ± 0.233	6.383 ± 0.713
Solvent control (2% d.H_2O)	96.383 ± 0.943	2.533 ± 0.935	1.083 ± 0.101	3.617 ± 0.943
C. edulis root water extract 1000 µg/ml	91.067 ± 0.361	6.467 ± 0.318	2.467 ± 0.217	8.933 ± 0.361
Curcumin 40 µM	57.120 ± 1.777	14.510 ± 1.544	27.600 ± 0.115	42.747 ± 1.685

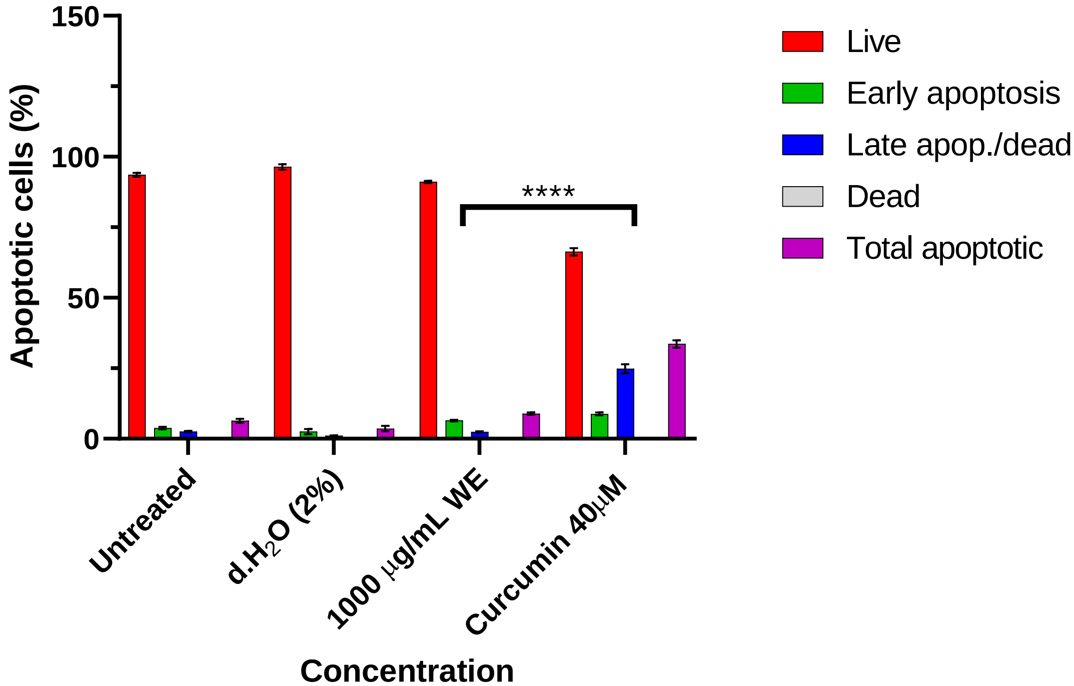

Figure 26: Average % apoptosis in *C. edulis* root water extract treated HeLa cells.

Treatment with C. edulis root water extract (1000 µg/ml) for 72 hours induced minimal apoptosis and curcumin (40 µM) for 24 hours significantly ($p \leq 0.0001$ ****) induced apoptosis as compared to untreated cells respectively.

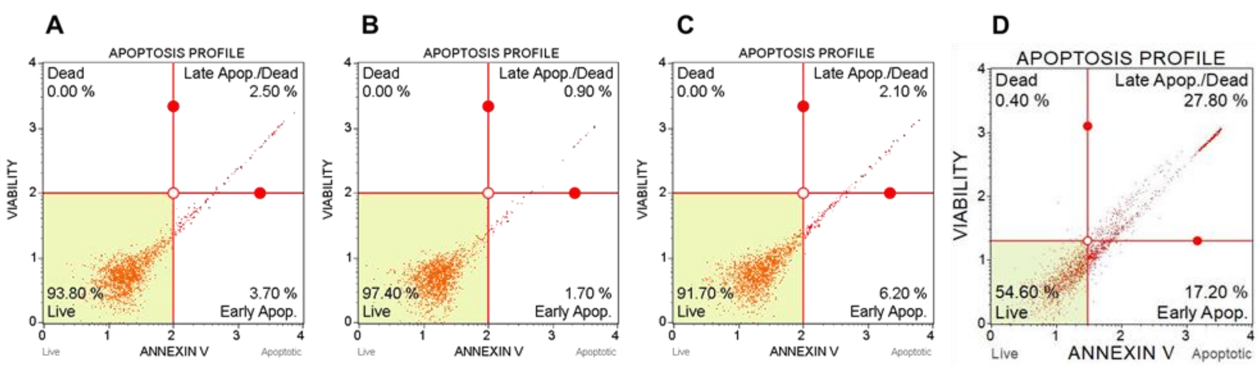

Figure 27: A-D: Confirmation of *C. edulis* root water extract induced minimal apoptosis in HeLa cells. The Muse™ Annexin V & Dead Cell apoptosis analysis confirmed that treatment with 1000 µg/ml of *C. edulis* root water extract (Figure 27 C) and 40 µM of the

positive control, curcumin (Figure 27 D) induced apoptosis in HeLa cells when compared with the untreated control (Figure 27 A) and solvent control in (Figure 27 B).

Table 20: Apoptosis average percentages ± SEM after the treatment of CaSki cells with *C. edulis* root water extract and curcumin.

Treatments	Mean (%) ± SEM			
	Live	Early apoptosis	Late apoptosis	Total apoptosis
Untreated	96.483 ± 0.660	1.583 ± 0.093	1.883 ± 0.543	3.467 ± 0.636
Solvent control (2% d.H_2O)	97.083 ± 1.025	1.483 ± 0.524	1.400 ± 0.477	2.883 ± 0.997
C. edulis root water extract 1000 µg/ml	91.983 ± 0.361	4.283 ± 0.468	3.667 ± 0.998	7.950 ± 1.422
Curcumin 40 µM	57.120 ± 1.777	14.510 ± 1.544	27.600 ± 0.115	42.747 ± 1.685

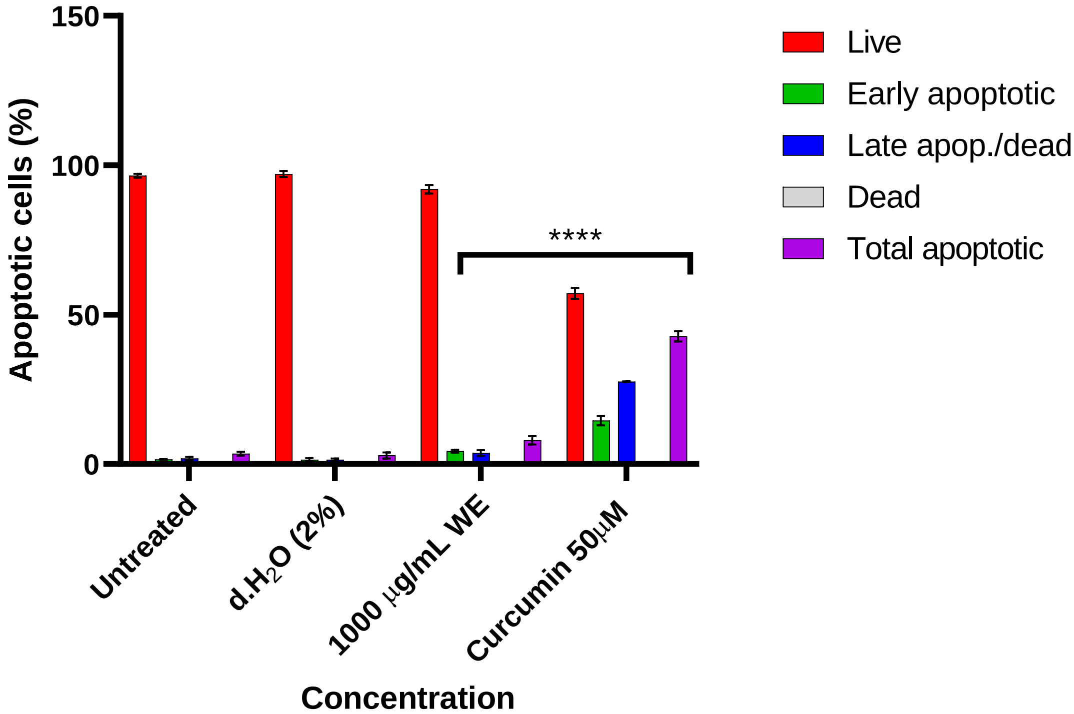

Figure 28: Average % apoptosis in *C. edulis* root water extract treated CaSki cells.

Treatment with *C. edulis* root water extract (1000 µg/ml) for 72 hours induced minimal apoptosis and curcumin (50 µM) for 24 hours significantly ($p \leq 0.0001$ ****) induced apoptosis as compared to untreated cells respectively.

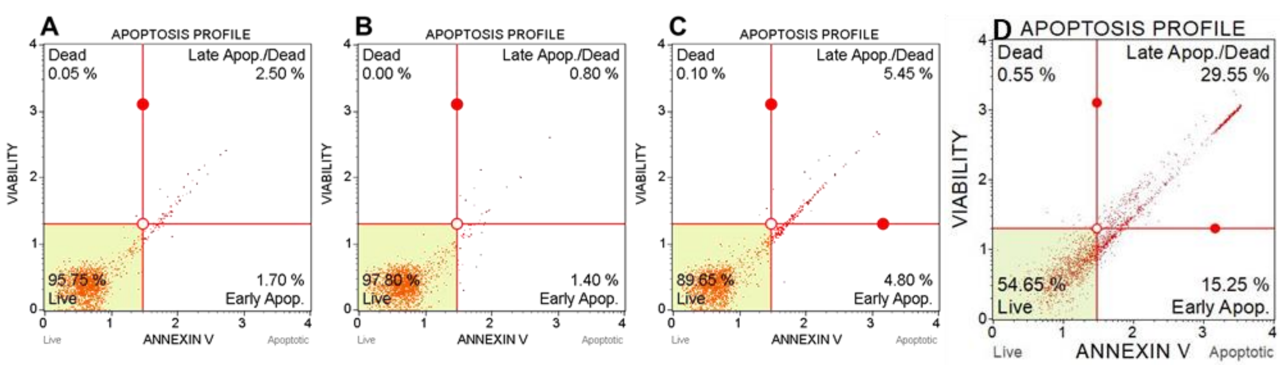

Figure 29: A-D: Confirmation of *C. edulis* root water extract induced minimal apoptosis in CaSki cells.

The Muse™ Annexin V & Dead Cell apoptosis analysis confirmed that treatment with 1000 µg/ml of *C. edulis* root water extract (Figure 29 C) and 50 µM of the positive control, curcumin (Figure 29 D) induced apoptosis in CaSki cells when compared with the untreated control (Figure 29 A) and solvent control in (Figure 29 B).

4.8. The effect of *C. edulis* root water extract on HeLa cells on human angiogenesis-related proteins

Every cell's nucleus contains deoxyribonucleic acid (DNA), which is used to store genetic information. Functioning proteins are produced by translating this genetic information from mRNA. The investigation of the expression patterns of angiogenesis proteins was conducted on HeLa cells using the human angiogenesis array kit (R&D Systems, Inc, USA). Following the preparation of cell lysates from cervical cancer cells (untreated, 1000 µg/ml of *C. edulis* root extract and 40 µM curcumin), each lysate was applied to an array membrane, incubated over the night, and the expression levels of 55 proteins linked to human angiogenesis were measured. **Table 21** and **Figure 30** display the expression density for the angiogenesis array's 55 proteins. The program Image J was used to assess the levels of protein expression, which were then shown as the mean pixel density standardised by the effects of the positive controls.

Table 21: Expression human angiogenesis proteins average in HeLa cells treated with *C. edulis* root water extract and curcumin

Human angiogenesis proteins	Mean (%) ± SEM
Angiopoietin-1	1959.333 ± 1009.509
Angiopoietin-2	2121.333 ± 882.141
Amphiregulin	2221.333 ± 924.852
Artemin	1928.000 ± 920.052
Coagulation factor III	18396.000 ± 3655.882
DPPIV	4971.000 ± 1428.746
EG-VEGF	2747.000 ± 1222.524
Endothelin-1	5283.000 ± 1207.195
GDNF	2407.667 ± 1444.138
GM-CSF	1911.000 ± 707.062
HB-EGF	9343.000 ± 1318.697
IGFBP-1	5321.000 ± 1600.733
IGFBP-2	12833 ± 2528.300
IL-8	14248.667 ± 2466.982
MMP-9	2397.000 ± 603.464
Pentraxin 3(PTX3)	2085.333 ± 357.157
PD-ECGF	1448.667 ± 459.047
Serpin E1	21876.667 ± 1575.647
TIMP-1	15565.333 ± 3998.188
TIMP-4	2266.333 ± 1057.021
Thrombospondin-1	4583.333 ± 765.614
uPA	15246.000 ± 4051.273
VEGF	6955.000 ± 105.105

NB: Mean (%) ± SEM of the entire table (single experiment).

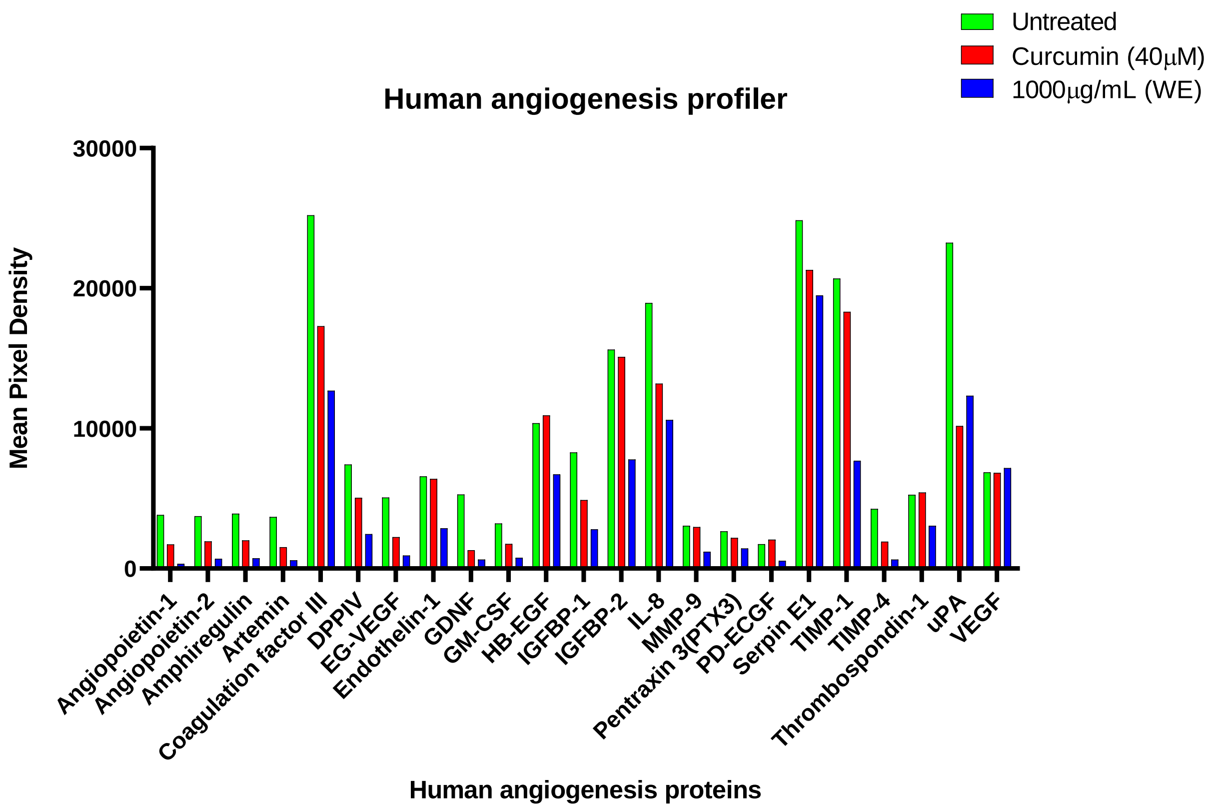

Figure 30: Confirmation of the effects *C. edulis* root water extract and curcumin in cervical cancer HeLa cells on downregulation and upregulation of human angiogenic proteins.

5. Introduction

For many years, medicinal plants have served as the basis for traditional treatments and are still developing innovative treatments for human use (Ren and Kinghorn, 2019) with little or non-existent adverse effects (Nwosu *et al.,* 2019). Researchers are considering medicinal plants as potential sources of anticancer medicines due to their richness in bioactive compounds, probably due to the fact that medicinal herbs are widely accessible, reasonably priced, and thought to have strong pharmacological effects (Akinyede *et al.,* 2020). Many medicinal plants are being utilized nowadays all over the world (Laka *et al.,* 2021), and the people of South Africa especially Pedi, Zulu and Xhosa use *C. edulis* as one of these plants to cure a wide range of illnesses (Akinyede *et al.,* 2020). There has been a lot of interest in using medicinal plants as potential medication candidates since many of their secondary metabolites, such as those found in *C. edulis*, are recognised to have a variety of advantageous qualities (Huang *et al.,* 2004). Our understanding of *C. edulis* anticancer properties is still lacking; therefore, the study was aimed at investigating its anticancer activities against cervical cancer cells.

5.1. Analysis of phytochemicals in *C. edulis* extracts

Secondary metabolites known as phytochemicals are in charge of many of the actions frequently connected to the application of medicinal plants for the management, treatment, and alleviation of a wide range of illnesses, including fatal conditions (Omoruyi *et al.,* 2012). Different solvents were used for extraction according to their polarity such as acetone, ethanol and water. The phytochemicals found in *C. edulis* extracts included proteins, tannins, flavonoids, phenols, saponins, steroids, coumarins, and carbohydrates. These findings are consistent with a 2021 study by Laka *et al.* on the screening of phytochemicals in medicinal plants. Wide-ranging biological activity of plant polysaccharides have been reported, including anti – tumour (Jia *et al.,* 2015), anti - oxidant (Yang *et al.,* 2015), anti - bacterial (Krichen *et al.,* 2015), anti-diabetic (Ferreira *et al.,* 2010), and wound repair properties (Lazareva *et al.,* 2002).

5.1.1. Qualitative analysis of *C. edulis* extracts

It was discovered that the juice extract included (tannins, flavonoids, steroids, coumarins, and carbs) in low amounts. The acetone, ethanol, and water extracts of *C. edulis* leaves revealed the presence of tannins, flavonoids, phenols, steroids, coumarins, proteins, and carbohydrates in different proportions. Tannins, flavonoids, phenols, saponins, steroids, coumarins, proteins, and carbohydrates were all found in significant amounts in the root extracts (acetone, ethanol, and water) of *C. edulis*.

To verify that *C. edulis* leaf and root extracts contained antioxidants, thin-layer chromatography (TLC) was used. Antioxidants were detected by TLC in extracts of leaves and roots under several mobile phases, including CEF, TEA, and EMW. Ready-to-use p-anisaldehyde, vanillin, and 0.2% 2,2-diphenyl-2-picryl-hyrazyl (DPPH) were sprayed over the TLC plates, respectively (Laka *et al.,* 2021). The TLC plates treated with DPPH displayed yellow spots, indicating the presence of antioxidants in the extracts of the leaves and roots, whereas P-anisaldehyde and vanillin revealed distinct colour bands, indicating the presence of antioxidants in the *C. edulis* extracts.

5.1.2. Quantitative analysis of *C. edulis* extracts

Polyphenol compounds have an antioxidant effect because of their ability to lower iron levels and scavenge free radicals (Nihal, 2007). The selection of extraction solvents can have a significant impact on the overall levels of polyphenols found in plants and their associated antioxidant activity.

5.1.2.1. Scavenging of free radicals by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)

The rich purple colour is caused by an unusual electron found in the DPPH free radicals (Gülçin, 2011). A quantitative measurement of the decolorization of DPPH can be obtained from changes in absorbance when it accepts an electron from an antioxidant molecule (Omoruyi *et al.,* 2012). In this research, this was seen as soon as the colour changed from purple to yellow. Acetone is capable of extracting a combination of polar as well as non-polar chemicals from plant samples on its own (Wintola and Afolayan, 2011). The polyphenol contents of *C. edulis* leaf extracts in aqueous, ethanol, acetone, and hexane solvents were recently compared. The optimum solvents for antioxidant capacity were shown to be ethanol and water (Sultana *et al.,* 2009). In this study, the ethanol solvent at 1000 µg/ml showed the highest DPPH inhibition in both leaves (65.337 ± 1.208) and roots at 250 µg/ml (75.420 ± 0.469).

5.1.2.2. Ferric reducing antioxidant power (FRAP)

Polyphenols are generally acknowledged to be important plant chemicals with a variety of biological functions, including antioxidant activity (Omoruyi *et al.,* 2012). Their ability to absorb and neutralize free radicals is largely attributed to their redox characteristics as donors of hydrogen and singlet oxygen quenchers (Gülçin *et al.,* 2011). Scientists, food makers, and consumers are becoming more interested in the role that plant materials' antioxidant contents play in maintaining health and preventing heart disease and cancer (Yazdanparast *et al.,* 2008). By monitoring the conversion of the Fe3+/ferricyanide complex to the Fe2+/ferrous form, the antioxidant activity of the *C. edulis* extracts was examined (Park and Jhon, 2010). In comparison to ascorbic acid (4153.073 ± 67.753), the leaves of *C. edulis* extracts showed the greatest reducing power at a concentration of 1000 µg/ml in the extracts of ethanol (4118.756 ± 204.925), acetone (3261.324 ± 363.379), juice (1897.928 ± 40.995), and water (1165.870 ± 55.795). The root extracts of *C. edulis* were shown to have the maximum reducing power in acetone (4422.065 ± 343.429), ethanol (2799.964 ± 99.501), and water (1793.361 ± 19.152) when compared to ascorbic acid (4153.073 ± 67.753). The present results are in full accordance with previous investigations that proposed a connection between plant extracts' potential to reduce phenolic content (Park *et al.,* 2010). This suggests that *C. edulis* may be a more effective natural antioxidant than common medications.

5.1.3. Chemical composition of *C. edulis* root acetone, ethanol and water extracts by Ultra-high performance liquid chromatography-Mass spectrophotometry (UHPLC-MS).

A high-resolution UHPLC–MS-based approach was used to examine the phytochemical variations in *C. edulis* root extracts. Three chemicals were identified in the acetone root extract, as table 7 illustrates. The extract from the roots of ethanol included thirteen components. The extract of water roots included eleven different chemicals. Both the acetone and the ethanol extracts included methyl stearate, of which the biological action is yet unclear. Additionally, ethanol and water extracts included artemin, whose biological action is still unclear. The sole known chemical in the other Aizoaceae family (*A. canariense*) is linolenic acid, which was only detected in the aqueous extract of *C. edulis* [Freije et al., 2013]. Linolenic acid is a substance that was used to treat inflammatory skin diseases including eczema and psoriasis.

5.2. Cytotoxic effects of *C. edulis* acetone, ethanol and water root extracts on Hek-293, HeLa and CaSki cells

C. edulis acetone and ethanol extract were promoting cell viability and proliferation in both HeLa and CaSki cells. Only *C. edulis* root water extract reduced cell viability and proliferation in dose-dependent manner in both HeLa and CaSki cells ($p \leq 0.0001$), hence IC_{50} was established at 1000 µg/ml after 72 hours of incubation. These results support the previous studies that revealed that the water extract of *C. edulis* reduced cell viability and proliferation in human colon cancer cells (HCT-116) after 24 hours of incubation (Hafsa *et al.,* 2016). The IC_{50} of *C. edulis* root water extract had no impact on non-cancerous (Hek-293) cells. Curcumin was used as a positive control and successfully reduced cell viability and proliferation after 24 hours in dose-depended manner and IC_{50} was found to be 40 µM in HeLa cells and 50 µM in CaSki cells ($p \leq 0.0001$) respectively.

5.3. Effects of *C. edulis* root water extract inducing apoptosis in HeLa and CaSki cells

Apoptosis, a regulated biological process that results in distinctive cellular alterations and necrosis, a sequence of morphological alterations in a fatally wounded cell, are the two recognized ways of cell death (Tang *et al.,* 2019). Cell shrinkage, nuclear fragmentation, and DNA fragmentation are among the cellular and nuclear morphological characteristics that define apoptotic cells. Following the manufacturer's instructions, the Muse™ Annexin V & Dead Cell Kit (Merck Millipore, Germany) was used to investigate the effects of *C.*

edulis water extract (WE) at 1000 µg/ml on the viability of HeLa and CaSki cells as determined by cell viability assays. On HeLa and CaSki cells, however, the *C. edulis* root water extract exhibited very little apoptotic impact. indicating that further research is necessary to determine whether the extracts caused another kind of cell death as shown in figure 7.1 and 7.2 respectively. Hence these results are incomparable since there are no published studies of the activity of *C. edulis* inducing apoptosis in cervical cancer.

5.4. The effect of *C. edulis* root water extract on HeLa cells in human angiogenesis-related proteins

Angiogenesis is the process of continuously recruiting and “pruning” blood vessels to cause extant blood vessels to sprout and remodel into a more complicated network of vasculature (Shim *et al.,* 2002). The process is complex and includes matrix degradation, peri-endothelial cell rearrangement, and endothelium growth, relocation, differentiation, and reconnection (Bicknell, 1994). Growth factors released by both stromal cells and the growing cancerous cells are examples of pro- and antiangiogenic factors that control the process and one such factor is vascular endothelial growth factor (VEGF), whose role in tumor angiogenesis has been well documented (Shim *et al.,* 2002). Only 23 of the 55 angiogenic proteins were expressed, as seen in Figure 30 and Table 21. Compared to cells treated with curcumin (40 µM), *C. edulis* root water extract (1000 µg/ml) treated cells had higher levels of urokinase type-plasminogen activator (uPA), and vascular endothelial growth factor (VEGF) was somewhat higher in *C. edulis* root water extract treated cells than in curcumin-treated and untreated cells. When compared to untreated cells, the expression of all 21 additional proteins were downregulated in *C. edulis* root water extract and curcumin, as seen in Table 21 and Figure 30. Upregulation of VEGF and uPA is associated with angiogenesis and cancer development, thus the slight increase in VEGF and uPA in this study support reported data by (Zhou *et al.,* 2021).

5.4.1. Expression of VEGF in treated HeLa cells with *C. edulis* root water extract

This study's finding of a little elevation of VEGF indicated that the *C. edulis* root extract had little effect on HeLa cells by triggering apoptosis-a marker of cancer dissemination.

Thus, blocking VEGF function decreases angiogenesis and lowers tumor growth *in vivo*. The majority of human malignancies investigated to far have been identified by activation of VEGF by cancer cells as well as upregulation of the receptors for VEGF on cancer-associated blood vessels (Dvorak *et al.,* 1995). Significantly, VEGF also seems to influence the formation of very early tumors and the development of skin squamous cell carcinomas (Skobe *et al.,* 1997).

5.4.2. Expression of uPA in treated HeLa with *C. edulis* root water extract

At the cell surface, plasminogen may be efficiently converted to plasmin due to the interaction between urokinase type-plasminogen activator (uPA) and its receptor, uPAR (Sun *et al.,* 2015). These proteins are therefore thought to be important regulators of invasion and metastasis (Dkhissi *et al.,* 2003). In this study, it was revealed that the slight expression of uPA concur with the expression of VEGF as the key mediators of early cancer progression. It has been documented that low-molecular-weight synthetic serine protease inhibitors and antibodies against uPA can stop the progression of cancer (Schmitt et al., 2011). It has also been discovered that blocking the production of plasmin may lessen the invasion and metastasis of cancer cells (Holst-Hansen *et al.,* 1996).

5.5. Conclusion

The management of many disorders is greatly aided by medicinal herbs. Scientists are still searching for medical plants that might provide plant-derived treatments as a potential source of drugs. Extracts from *C. edulis* seem to include components with antioxidant action. The current study has shown that ethanol and acetone were the best solvents for antioxidant activity. The antioxidant capacity and DPPH inhibitory activity of *C. edulis* roots were found to be higher than in the leaves. The root water extract of *C. edulis* was cytotoxic to HeLa and CaSki cells, while the Hek-293 (non-cancerous), cells were not affected. The *C. edulis* acetone and ethanol root extracts did not show any cytotoxic activity on HeLa and CaSki cells. The *C. edulis* root water extract increased slightly the levels of VEGF and uPA proteins, which cause angiogenesis, but had no effect on HeLa or CaSki cell death. In conclusion, extracts from *C. edulis* should be studied further in relation to alternative modes of cell death, cervical cancer metastasis and drug resistance, in addition to the production of uPA and VEGF proteins.

5.6. Future work

The cytotoxic effects of *C. edulis* root water extract on HeLa and CaSki cells were observed in this investigation. Determining which biochemical route is involved and evaluating the impact of *C. edulis* root water extract on the cell cycle using bioinformatics tools will be intriguing. Additionally, investigate the potential therapeutic effects of all unidentified compounds included in acetone, ethanol, and water extracts and drug resistance should be investigated.

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Appendices

Appendix A: Cell count & viability Storage Conditions

• Store the Muse® Count & Viability

Reagent at 2 to 8°C, protected from light.

Kit Components

Muse® Count & Viability Reagent:

• Part No. 4000-0335, 40 mL

• Part No. 4000-0340, 240 mL

Materials Recommended

• Guava® Muse® Cell Analyzer

• Cell suspension

• Dilution buffer: Phosphate buffered

saline (PBS), or equivalent balanced salt

solution (pH 7.2 to 7.4), or complete

growth medium

• Micropipettes

• Disposable micropipette tips

• Microcentrifuge tubes with screw caps,

1.5 mL (VWR Catalog No. 16466-030, or equivalent)

• Muse® Count & Viability Cell Dispersal

Reagent (Catalog No. MCH100107).

Appendix B: Annexin V apoptosis kit

Storage Conditions

Store the Muse™ Annexin V & Dead Cell Kit

at 2 to 8°C, protected from light.

Kit Components

Muse™ Annexin V & Dead Cell Reagent

(Part No. 4700-1485, 100 tests/bottle)

Materials Recommended

• Muse™ Cell Analyzer

• Cell suspension, untreated and treated

to undergo apoptosis

• Micropipettes

• Disposable micropipette tips

• Microcentrifuge tubes with screw

caps, 1.5 mL (VWR Catalog No.

16466-030, or equivalent)

• Muse™ Cell Dispersal Reagent

(Catalog No. MCH100107), optional

• Vortex mixer

• 1% bovine serum albumin (BSA),

1% foetal bovine serum (FBS), or

10% normal human serum (NHS)

Appendix C: Human angiogenesis proteome profiler

MATERIALS PROVIDED & STORAGE CONDITIONS

Store the unopened kit at 2-8 °C. Do not use past kit expiration date.

PART	PART #	DESCRIPTION	STORAGE OF OPENED/ RECONSTITUTED MATERIAL
Human Angiogenesis Array	893313	4 nitrocellulose membranes each containing 55 different capture antibodies printed in duplicate.	Return unused membranes to the foil pouch containing the desiccant pack. Reseal along entire edge of the zip-seal. May be stored for up to 3 months at 2-8 °C.*
Array Buffer 4	895022	21 mL of a buffered protein base with preservatives. *May contain a precipitate. Mix well before and during use.*	May be stored for up to 3 months at 2-8 °C.*
Array Buffer 5	895876	21 mL of a buffered protein base with preservatives.	

Array Buffer 7	895924	21 mL of a buffered protein base with preservatives.	
Wash Buffer Concentrate	895003	2 vials (21 mL/vial) of a 25-fold concentrated solution of buffered surfactant with preservative. *May turn yellow over time.*	
Detection Antibody Cocktail, Human Angiogenesis Array	893312	1 vial of biotinylated antibody cocktail; lyophilized.	
Streptavidin-HRP	893019	200 µL of streptavidin conjugated to horseradish-peroxidase.	
Chemi Reagent 1	894287	1 vial (2.5 mL).	
Chemi Reagent 2	894288	1 vial (2.5 mL).	
4-Well Multi-dish	607544	Clear 4-well rectangular multi-dish.	Store at room temperature.
Transparency Overlay Template	607586	1 transparency overlay template for coordinate reference.	

REAGENT PREPARATION

Bring all reagents to room temperature before use.

Human Angiogenesis Array - Four nitrocellulose membranes each containing 55 different capture antibodies printed in duplicate. **Handle membranes only with gloved hands and flat-tipped tweezers.**

Detection Antibody Cocktail - One vial of lyophilized biotinylated antibodies. Before use, reconstitute the Detection Antibody Cocktail with 100 μL of deionized or distilled water.

1X Wash Buffer - If crystals have formed in the concentrate, warm the bottles to room temperature and mix gently until the crystals have completely dissolved. Add 40 mL of Wash Buffer Concentrate to 960 mL of deionized or distilled water to prepare 1000 mL of 1X Wash Buffer.

Chemi Reagent Mix - Chemi Reagent 1 and 2 should be mixed in equal volumes within 15 minutes of use. **Protect from light. 1 mL of resultant mixture is required per membrane.**

ARRAY PROCEDURE

Bring all reagents to room temperature before use. Keep samples on ice. To avoid contamination, wear gloves while performing the procedures.

1. Prepare all reagents and samples as directed in the previous sections.

2. Pipette 2 mL of Array Buffer 7 into each well of the 4-Well Multi-dish to be used. Array Buffer 7 serves as a block buffer.

3. Using flat-tip tweezers, remove each membrane to be used from between the protective sheets and place in a well of the 4-Well Multi-dish. The number on the membrane should be facing upward.

Note: *Upon contact with Array Buffer 7, the blue dye from the spots will disappear, but the capture antibodies are retained in their specific locations.*

4. Incubate for one hour on a rocking platform shaker. Orient the tray so that each membrane rocks end to end in its well.

5. While the membranes are blocking, prepare samples by adding up to 1 mL of each sample to 0.5 mL of Array Buffer 4 in separate tubes. Adjust to a final volume of 1.5 mL with Array Buffer 5 as necessary.

6. Add 15 µL of reconstituted Detection Antibody Cocktail to each prepared sample. Mix and incubate at room temperature for one hour.

7. Aspirate Array Buffer 7 from the wells of the 4-Well Multi-dish and add sample/antibody mixtures prepared in steps 5 and 6. Place the lid on the 4-Well Multi-dish.

8. Incubate overnight at 2-8 °C on a rocking platform shaker.

Note: *A shorter incubation time may be used if optimal sensitivity is not required.*

9. Carefully remove each membrane and place into individual plastic containers with 20 mL of 1X Wash Buffer. Rinse the 4-Well Multi-dish with deionized or distilled water and dry thoroughly.

10. Wash each membrane with 1X Wash Buffer for 10 minutes on a rocking platform shaker. Repeat two times for a total of three washes.

11. Dilute the Streptavidin-HRP in Array Buffer 5 using the dilution factor on the vial label. Pipette 2.0 mL of diluted Streptavidin-HRP into each well of the 4-Well Multi-dish.

12. Carefully remove each membrane from its wash container. Allow excess buffer to drain from the membrane. Return the membrane to the 4-Well Multi-dish containing the diluted Streptavidin-HRP. Cover the wells with the lid.

13. Incubate for 30 minutes at room temperature on a rocking platform shaker.

14. Wash each array as described in steps 9 and 10.

Note: *Complete the remaining steps without interruption.*

15. Carefully remove each membrane from the wash container. Allow excess Wash Buffer to drain from the membrane by blotting the lower edge onto paper towels. Place each membrane on the bottom sheet of a plastic sheet protector with the identification number facing up.

16. Pipette 1 mL of the prepared Chemi Reagent Mix evenly onto each membrane.

Note: *Using less than 1 mL of Chemi Reagent Mix per membrane may result in incomplete membrane coverage.*

17. Carefully cover with the top sheet of the plastic sheet protector. Gently smooth out any air bubbles and ensure Chemi Reagent Mix is spread evenly to all corners of each membrane. Incubate for 1 minute at room temperature.

18. Position paper towels on the top and sides of the plastic sheet protector containing the membranes and carefully squeeze out excess Chemi Reagent Mix.

19. Remove the top plastic sheet protector and carefully lay an absorbent lab wipe on top of the membranes to blot off any remaining Chemi Reagent Mix.

20. Leaving membranes on the bottom plastic sheet protector, cover the membranes with plastic wrap taking care to gently smooth out any air bubbles. Wrap the excess plastic wrap around the back of the sheet protector so that the membranes and sheet protector are completely wrapped.

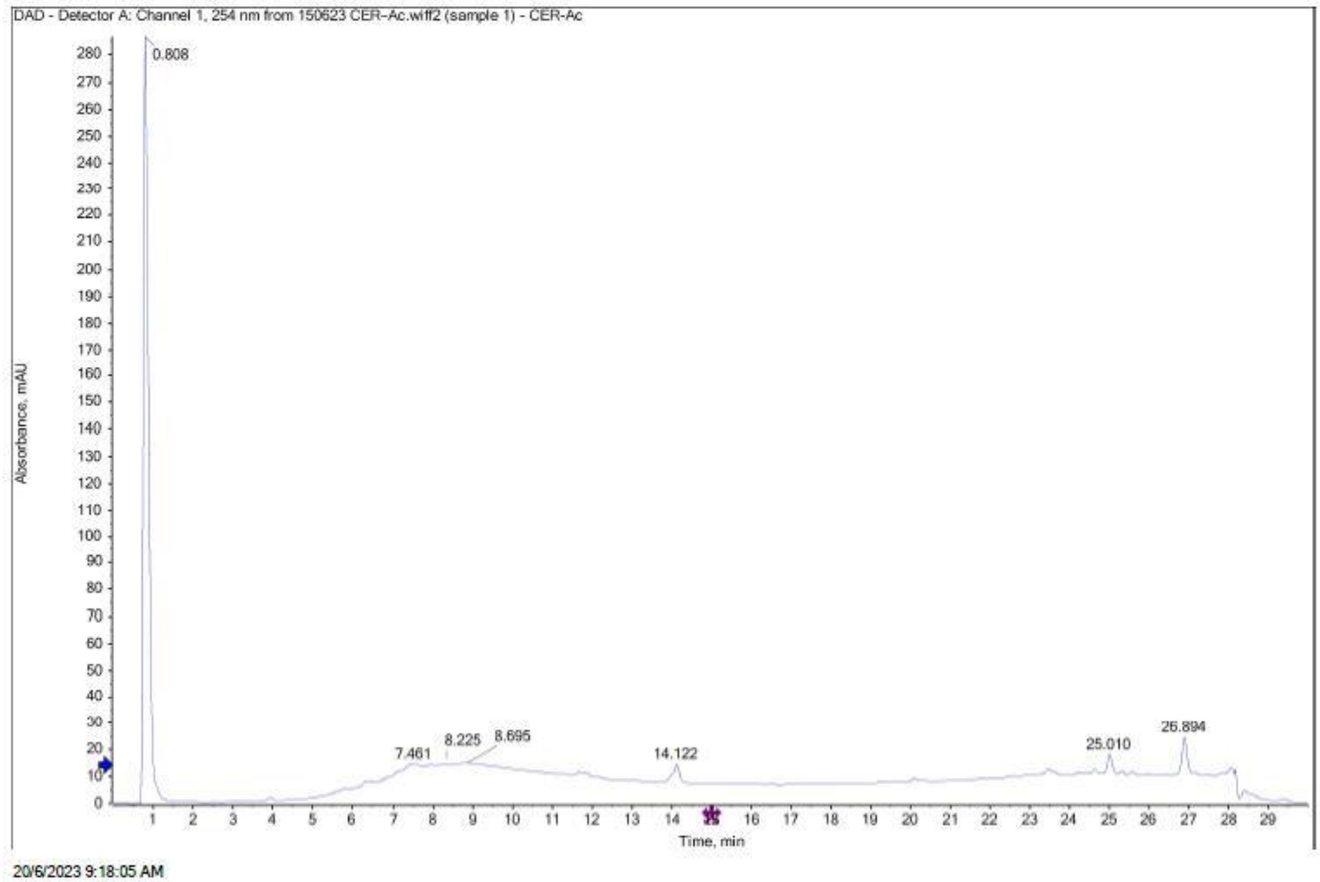
21. Place the membranes in an autoradiography film cassette with the identification numbers facing up.

Note: *Use an autoradiography cassette that is not used with radioactive isotope detection.*

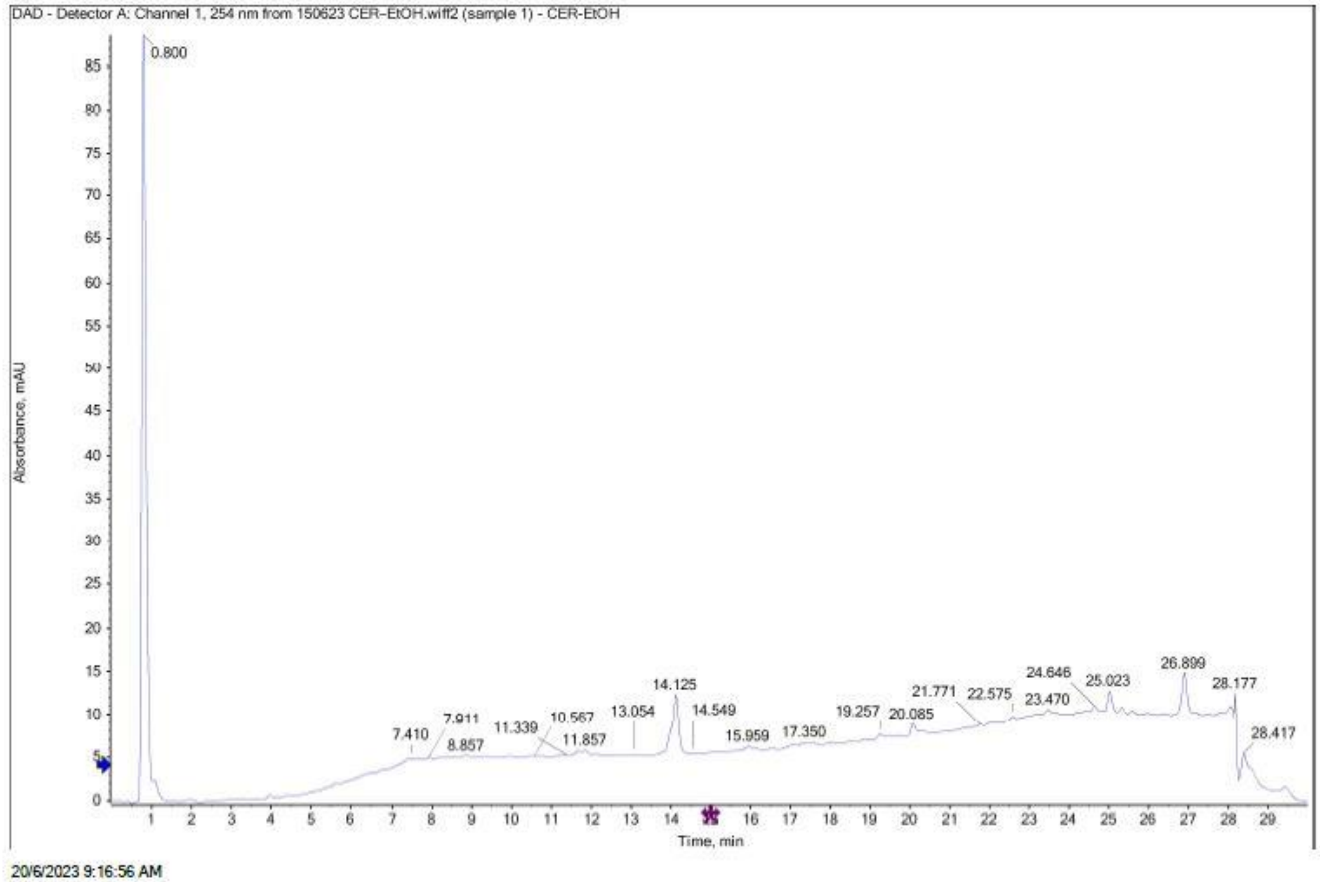
22. Expose membranes to X-ray film for 1-10 minutes. Multiple exposure times are recommended.

Appendix D: Ultra-high performance liquid chromatography-Ultra-violet/Diode array detector (UHPLC-UV/DAD) chromatographs for acetone, ethanol and water extracts.

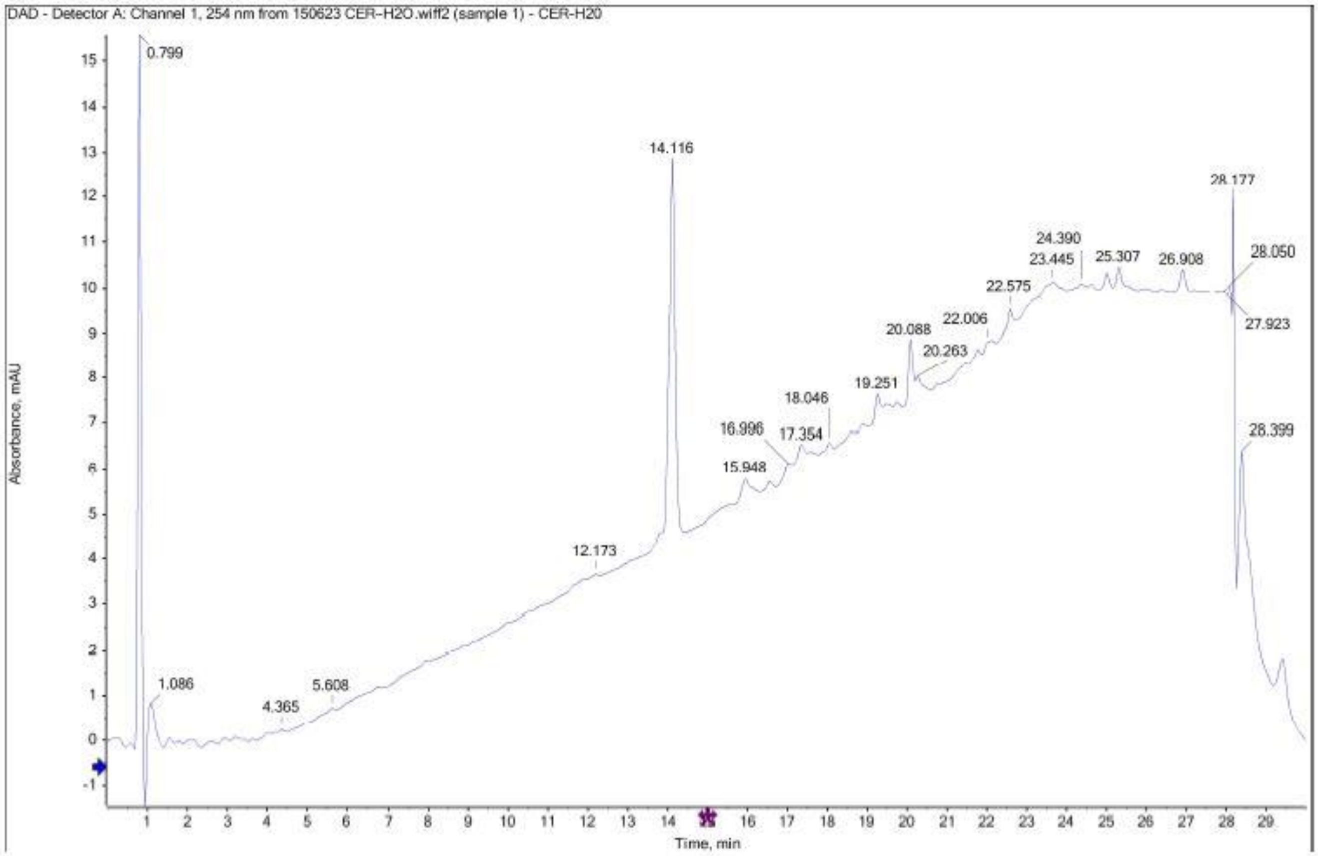
C. edulis acetone extract chromatograph:



C. edulis ethanol extract chromatograph:



C. edulis water extract chromatograph:



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